Detection of *SCN1A* mutations in patients with severe myoclonic epilepsy in infancy by custom resequence array

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SUMMARY

Introduction. Very few epilepsy phenotypes have been associated with causative genes; nevertheless, it is becoming possible, for some epilepsy phenotypes, to predict the most efficacious anti-epileptic drugs for patients based on their genetic makeup. The development of individualized medicine based on genetic information and the genetic diagnosis of epilepsy are expected to greatly improve the diagnosis and treatment of epilepsy. Here, we developed a DNA array (resequencing array) for the genetic diagnosis of epilepsies in which 14 epilepsy – related genes (*SCN1A, SCN1B, CHRNA4, CHRNA7, CHRNB2, GABRA1, GABRD, GABRG2, CACNB4, CLCN2, KCNQ2, KCNQ3, CACNA1A,* and *CACNA1H*) have been mounted.

Aim. The aim of the present study is to evaluate the performance of our custom array in detecting the *SCN1A* mutations in patients with severe myoclonic epilepsy in infancy.

Material and methods. We compared mutation data generated by DNA array sequencing of DNA samples from patients with severe myoclonic epilepsy in infancy to the data generated by capillary sequencing.

Results. Heterozygosity was detected in 44 of 48 patients (92%). We successfully identified epilepsy mutations, and the results of DNA array analyses were largely consistent with the results of capillary sequencing analysis.

Conclusion. These findings indicate that this DNA array is likely to be a useful tool in clinical settings.

Key words: DNA chip • epilepsy • resequence • *SCN1A* • SMEI

Original paper

BACKGROUND

Since the first responsible gene for idiopathic epilepsy, *CHRNA4*, was identified in a patient with autosomal dominant nocturnal frontal lobe epilepsy in 1995 (Steinlein et al., 1995), at least a further 25 genes have been identified as epilepsy-related genes (Reid et al., 2009; Ottman et al., 2010). Most encode voltage-gated ion channels (Na^+, K^+, Ca^{2+}, and Cl^-) or ligand-gated channels (nicotinic acetylcholine and GABAA receptors). Causative mutations in these genes include missense/nonsense mutations that result from single-base substitution or deletion/insertion frame-shift mutations. Microchromosomal deletions that cause the loss of multiple exons or the loss of a whole gene were also recently found to be associated with severe myoclonic epilepsy in infancy (SMEI) (Madia et al., 2006; Mulley et al., 2006; Suls et al., 2006; Wang et al., 2008; Saitoh et al., 2012). These various mutations are thought to cause some cases of idiopathic epilepsies, whereas other cases of epilepsy are caused by mutation of only a single gene. Identification of mutated genes or loci in individual patients is an important step toward our ultimate aim, which is the development of individualized pharmacotherapy for epilepsy.

The current treatment of epilepsy is mainly pharmacotherapy with anti-epileptic drugs (AEDs), but this treatment is symptomatic and not a radical treatment. In general, the selection of AEDs is based on the types of seizures and trial and error, and 30% of patients with epilepsy do not achieve adequate seizure control from AED treatment (Kwan and Brodie, 2000). The aim of individualized medicine for epilepsy is to control seizures by creating customized treatment approaches for each patient based on the patient's genetic makeup This individualized approach is based on the belief that genetic information can be used to improve the selection of an effective AED and assist in the determination of an appropriate dose. It is known that some genes cause pharmacoresistance (Nasir et al., 2011). However, the identification of genes associated with epilepsy is ongoing work, and it is obvious that more epilepsy-related genes will be identified. Therefore, more efficient and cost-effective tools for the detection of individual known mutations in patients with epilepsy are needed.

AIM

Here, we describe a custom resequencing array that can be used for high-throughputdetection of epilepsy-related mutations. This array can enable researchers and clinicians to detect both known and novel missense/nonsense mutations. The aim of the present study is to evaluate the performance of this custom array. We investigated the consistency of this array by comparing results of resequencing *SCN1A* mutations (missense or nonsense mutations) with results from capillary sequencing; all DNA samples were taken from patients with SMEI.

MATERIAL AND METHODS

Sample information

We investigated 89 patients with SMEI. Blood samples were collected from 46 SMEI patients who had missense or nonsense mutations of *SCN1A* that were identified by capillary sequencing. The array analyst was blind to the capillary sequencing data. Genomic DNA was extracted from peripheral white blood cells according to standard protocols. Two patients, S41 and S73, each carried two mutations in *SCN1A*; therefore, the total number of mutations was 48 (Table 1). Patients with SMEI develop apparently normally until their infancy, when the first seizure episode often is seen as generalized or unilateral tonic-clonic or clonic seizures induced by fever. The first attack of SMEI may be discernible from simple febrile seizures, and the seizure activity evoked by fever tends to be prolonged and could advance to status epilepticus. Patients subsequently manifest a variety of seizure types, including atypical absence, myoclonic, and simple or complex partial seizures, which are generally refractory. Seizures induced easily by high- and low-grade fever are common. Around the second year of life, psychomotor delay becomes evident. Febrile seizures and/or epilepsy are often seen in the family. Diagnosis was achieved according to well-described diagnosis criteria (Dravet et al., 1992; Fukuma et al., 2004). Patients were diagnosed as having SMEI by epileptologists and neuropediatricians and registered with the Epilepsy Genetic Study Group, Japan. Informed consent was obtained from the parents of each patient with SMEI. This study was approved by the Ethics Committee of each participating institution.

Probe design for custom resequencing array

Custom-designed resequencing microarrays consist of a high density of 25-mer oligonucleotide probes synthesized on the array by photolithography and solid-phase DNA synthesis for hybridization-based analysis of spe-

Table 1. Comparison of resequencing results on *SCN1A* mutations with results from capillary sequencing data

Sample No.	Mutation	Results
S1	c.4547C>A	C
S4	c.4064T>C	C
S6	c.2837G>A	C
S7	c.5734C>T	C
S8	c.302G>A	C
S10	c.2134C>T	C
S12	c.5674C>T	C
S14	c.2802G>C	I
S15	c.568T>C	C
S16	c.2855G>A	C
S21	c.2802G>A	C
S25	c.2134C>T	C
S26	c.2831T>C	C
S30	c.2243G>A	I
S31	c.2134C>T	C
S34	c.3373G>T	I
S35	c.4384T>C	C
S38	c.4280A>C	C
S39	c.4573C>T	C
S41	c.251A>G	C
	c.4723C>T	C
S42	c.4664T>G	C
S47	c.1277A>G	C
S50	c.4032T>G	C
S53	c.4633A>G	C
S54	c.5734C>T	C
S55	c.1150T>C	C
S58	c.4573C>T	C
S59	c.3075T>G	I
S60	c.2584C>T	C
S62	c.2134C>T	C
S63	c.4507G>A	C
S64	c.301C>T	C
S69	c.251A>G	C
S70	c.2819C>T	C
S72	c.4358A>G	C
S73	c.680T>G	C
	c.4723C>T	C
S74	c.5051A>C	C
S76	c.4823A>G	C
S78	c.1087A>C	C
S79	c.5542C>T	C
S80	c.535T>C	C
S82	c.4679T>A	C
S84	c.5674C>T	C
S86	c.1277A>G	C
S87	c.58G>T	C
S88	c.1150T>C	C

C=consistent; I=inconsistent

cific sequences of interest (Hacia, 1999). Affymetrix technology demonstrates high performance in microarray quality control study (coefficient variations was under 10% within facility) (Shi et al., 2006). This custom resequencing array (169 format) was designed for detecting mutations from patients with a diagnosis of epilepsy. Based on previous reports of mutation information from patients with epilepsy, sequences from 54 regions of 14 epilepsy-related genes (*SCN1A, SCN1B, CHRNA4, CHRNA7, CHRNB2, GABRA1, GABRD, GABRG2, CACNB4, CLCN2, KCNQ2, KCNQ3, CACNA1A,* and *CACNA1H*) were tiled on the array; the first and last 12 flanking bases of each region were included in the sequences. Table 2 lists the names, regions, and lengths of the tiled gene sequences; the NCBI accession numbers; and the locus start and end sites. Repetitive regions, which lead to cross hybridization, were removed by using RepeatMasker (http://www.repeatmasker.org/). Mutations in *SCN2A* and *CHRNA2*, which are also candidate epilepsy-related genes, were found in exons 2, 4, 5, 15, 16, 20, 21, 26, and 27 of *SCN2A* and exon 6 of *CHRNA2* in DNA samples from the patients with epilepsy. However, these two genes share highly homologous sequences with *SCN1A* and *CHRNA4*, respectively; therefore *SCN2A* and *CHRNA2* were excluded from the array to avoid cross hybridization. The sequences arrayed on the chip consisted of 17,580 bp and represented approximately 91% of all known epilepsy-related regions. In addition, 38 to 47 bp of sequences representing 24 known insertion or deletion sites were tiled on the array to detect known deletion/ insertion mutations that cause epilepsy. Table 3 lists the sites, sequences, and lengths of each deletion/ insertion. We designated this custom-designed array as the *EpiGene*.

Primers and PCR conditions

PCR primers were designed to amplify each sequence tiled on the array. As shown in Table 4, PCR was performed with 48 primer sets under five PCR conditions. For conditions 1–4, the reaction consisted of 0.3 U KOD FX (Toyobo), 15 ng of genomic DNA, 7.5 µL of 2x KOD FX buffer, 3 µL of dNTPs (2 mM), and 6 pmol of each primer for a total reaction volume of 15 µL. For condition 5, the reaction consisted of 0.6 U KOD Plus (Toyobo), 3 µL of 10x buffer, 3 mL of dNTPs (2 mM), 12 pmol of each primer, and 30 ng of genomic DNA for a total reaction volume of 30 µL. All PCRs began with a 2-min incubation at 94°C to denature the duplexes;

Table 2. Probe information 1

Gene	Sequence region tiled on the array	Length (bp)	NCBI Accession Number	Locus (start)	Locus (end)
Sodium channel	SCN1A exon1	290	NT_005403.16	17139562	17139273
Voltage-gated, type I, alpha subunit	SCN1A exon2	145	NT_005403.16	17124629	17124485
	SCN1A exon3	116	NT_005403.16	17122428	17122313
	SCN1A exon4	155	NT_005403.16	17120707	17120553
	SCN1A exon5	118	NT_005403.16	17118884	17118767
	SCN1A exon6	296	NT_005403.16	17117929	17117634
	SCN1A exon7	90	NT_005403.16	17114890	17114801
	SCN1A exon8,intron8 and exon9	1025	NT_005403.16	17113709	17112685
	SCN1A exon10	311	NT_005403.16	17111268	17110958
	SCN1A exon11	407	NT_005403.16	17109990	17109584
	SCN1A exon12	159	NT_005403.16	17108365	17108207
	SCN1A exon13	265	NT_005403.16	17107410	17107146
	SCN1A exon14	200	NT_005403.16	17105537	17105338
	SCN1A exon15	383	NT_005403.16	17104073	17103691
	SCN1A exon16	509	NT_005403.16	17102471	17101963
	SCN1A exon17	147	NT_005403.16	17081668	17081522
	SCN1A exon18 and 5bp of intron18 5'-region	186	NT_005403.16	17079839	17079654
	SCN1A exon19	200	NT_005403.16	17078223	17078024
	SCN1A exon20	149	NT_005403.16	17075782	17075634
	SCN1A exon21	308	NT_005403.16	17068694	17068387
	SCN1A exon22 and 1bp of intron22 5'-region	80	NT_005403.16	17065717	17065637
	SCN1A exon23	164	NT_005403.16	17064116	17063953
	SCN1A exon24	131	NT_005403.16	17062058	17061928
	SCN1A exon25	298	NT_005403.16	17060357	17060061
	1197bp of SCN1A exon26 5'-region	1197	NT_005403.16	17058363	17057167
	2084bp of SCN1A exon26 3'-region	2084	NT_005403.16	17057162	17055078
Sodium channel, voltage-gated, type I, beta subunit	SCN1B exon3	267	NT_011109.15	7792608	7792874
Neuronal nicotinic acetylchorine receptor α4 subunit	CHRNA4 exon5	1401	NT_011333.5	719022	717622
Neuronal nicotinic acetylchorine receptor α7 subunit	CHRNA7 exon10	545	NT_010194.16	3250685	3251229
Neuronal nicotinic acetylchorine receptor β2 subunit	CHRNB2 exon5	999	NT_004487.18	5034007	5035005
γ-aminobutyric acid receptor subtype A (GABAA) α1 subunit	GABRA1 exon10	229	NT_023133.12	6132217	6132445
γ-aminobutyric acid receptor subtype A (GABAA) δ subunit	GABRD exon5	109	NT_004350.18	1437632	1437740
	GABRD exon6	164	NT_004350.18	1438210	1438373
γ-aminobutyric acid receptor subtype A (GABAA) γ2 subunit	GABRG2 exon2	178	NT_023133.12	6330379	6330556
γ-aminobutyric acid receptor subtype A (GABAA) γ2 subunit	GABRG2 exon4	247	NT_023133.12	6334189	6334435
Calcium channel, voltage-dependent, beta 4 subunit	CACNB4 exon3	146	NT_005403.16	2949315	2949170
	CACNB4 exon13	212	NT_005403.16	2908033	2907822
Cloride channel 2	CLCN2 exon5	160	NT_005612.15	90571046	90570887
	CLCN2 exon19	100	NT_005612.15	90565761	90565662
Potassium voltage-gated channel, KQT-like subfamily, member 2	105bp of KCNQ2 exon1 5'- region	105	NT_011333.5	840634	840530
	361bp of KCNQ2 exon1 3'-region	361	NT_011333.5	840499	840138
	KCNQ2 exon2	117	NT_011333.5	814833	814717
	KCNQ2 exon8	121	NT_011333.5	801899	801779
Potassium voltage-gated channel, KQT-like subfamily, member 3	KCNQ3 exon5	182	NT_008046.15	46406047	46405866
Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	CACNA1A exon3	166	NT_011295.10	4826644	4826479
	CACNA1A exon33	92	NT_011295.10	4608903	4608812
	CACNA1A exon36	154	NT_011295.10	4603838	4603685
Calcium channel, voltage-dependent, T type, alpha 1H subunit	CACNA1H exon4	160	NT_037887.4	1185420	1185579
	CACNA1H exon7	342	NT_037887.4	1190244	1190585
	368bp of CACNA1H exon9 5'-region	368	NT_037887.4	1191651	1192018
	437bp of CACNA1H exon9 3'-region	437	NT_037887.4	1192029	1192466
	CACNA1H exon10	475	NT_037887.4	1193998	1194472
	CACNA1H exon11	178	NT_037887.4	1195102	1195279
	CACNA1H exon23	152	NT_037887.4	1201469	1201620

Table 3. Probe information 2 (deletion/insertion)

Insertion/Deletion mutation site	Length (bp)	†Sequence (5'-3')	Reference
CHRNA4_ins776GCT	47	GCTGTCGCTCACCGTCTTCCTGCTGCTCGCTATCACCGAGATCATCC	(Steinlein et al., 1997)
KCNQ2_del314TCC	38	CGTGTTCCTCCTGGTTTTC*TGCCTCGTGCTGTCTGTGT	(Claes et al., 2004)
SCN1A_del429GT	42	CACTATTTTGACAAACT*GTGTTTATGACAATGAGTAACCCTC	(Fukuma et al., 2004)
SCN1A_del657AG	42	CTCGGCATTGAGAACATTCAG*TTCTCCGAGCATTGAAGACGA	(Claes et al., 2001)
SCN1A_del853CTTC	42	TACAATGGCCTCCCACCAATGCTT*GGAGGAACATAGTATAGA	(Nabbout et al., 2003)
SCN1A_del1121C	42	CCTTCAGTTGGGCTTTTTTGTC*TTGTTTCGACTAATGACTCA	(Nabbout et al., 2003)
SCN1A_del1502G	42	CTAAGGAAAGAAGAAATCGGAG*AAGAAAAGAAAACAGAAAGA	(Ohmori et al., 2002)
SCN1A_ins1640A	43	GAACCGATTGACATATGAAAAAGAGGTACTCCTCCCCACACCA	(Ohmori et al., 2002)
SCN1A_del1820C	42	ATAACGAGAGCCGTAGAGATTC*TTGTTTGTGCCCCGACGACA	(Ohmori et al., 2002)
SCN1A_ins2118AA	44	TCCATGGACTTTCTAGAAGATAACCTTCCCAAAGGCAACGAGCA	(Kearney et al., 2006)
SCN1A_del2740A	42	GTCGGCATGCAGCTCTTTGGTAAA*GCTACAAAGATTGTGTCT	(Kearney et al., 2006)
SCN1A_del2835C	42	CCACTCCTTCCTGATTGTGTTC*GCGTGCTGTGTGGGGAGTGG	(Fujiwara et al., 2003)
SCN1A_del3195TA	42	AGACAGTTGTATGTCCAATCA*CAGCAGAAATTGGGAAAGATC	(Nabbout et al., 2003)
SCN1A_ins3298AA	44	AAAAATACATTATTGATGAAAAAGTGATTACATGTCATTCATAA	(Claes et al., 2001)
SCN1A_del3524TT	42	TAGTGGAACCTGAAGAAACTC*GAACCAGAAGCTTGTTTCACT	(Fukuma et al., 2004)
SCN1A_ins3643TA	43	AAGGACGTGTTTCCGAATAGTATTGAACATAACTGGTTTGAGA	(Nabbout et al., 2003)
SCN1A_del3864CTT	42	CAATGCCTGGTGTTGGCTGGAC*TTAATTGTTGATGTTTCATT	(Ohmori et al., 2002)
SCN1A_del5010GTTT	42	GATGATGTCCCTTCCTGCG*TTAACATCGGCCTCCTACTCTTC	(Claes et al., 2001)
SCN1A_ins5239A	44	GACTGTGACCCTAATAAAGTTAAACCCTGGAAGCTCAGTTAAGG	(Gennaro et al., 2003)
SCN1A_ins5291T	43	GAACCCATCTGTTGGAATTTTTCTTTTTTGTCAGTTACATCAT	(Fujiwara et al., 2003)
SCN1A_del5414TT	42	AGCCTCTGAGTGAGGATGACT*GAGATGTTCTATGAGGTTTGG	(Nabbout et al., 2003)
SCN1A_del5657G	42	GTGGAGAGATGGGATGCTCTAC*AATACAGATGGAAGAGCGATT	(Nabbout et al., 2003)
SCN1A_del5668G	42	GATGCTCTACGAATACAGATG*AAGAGCGATTCATGGCTTCCA	(Nabbout et al., 2003)
SCN1A_del5711ATCA	42	CCAATCCTTCCAAGGTCTCCT*GCCAATCACTACTACTTTAAA	(Sugawara et al., 2001)

†*=deletion site; _=insertion site

the denaturation was followed by 30 cycles of each condition shown in Table 4. PCR products were subjected to electrophoresis on a 1% agarose gel to confirm that bands of the appropriate sizes were present.

Quantitation, pooling, and fragmentation

The following steps were performed according to the manufacturer's instructions. To pool each PCR product with other products of the same molecular weight (111.7 pM/array), PCR products were quantitated by using a Quant-iT™ PicoGreen dsDNA Assay kit (Invitrogen) and a microplate reader (MTP-601Lab, Corona). Pooled samples were purified by using the DNA Amplification Clean-Up Kit (Clontech), and 230-ng pooled samples were fragmented with a fragmentation reagent (Affymetrix). Fragmentation reaction mixtures were incubated at 37°C for 35 min to let the reactions proceed, and then at 95°C for 15 min to inactivate the fragmentation reagent. Samples of fragmented DNA (2 μL each) were subjected to electrophoresis on 20% TBE polyacrylamide gels to ensure that an optimal range of fragment sizes (20 to 200 bp) was achieved.

Labeling and hybridization

Fragmented samples were labeled with biotin at the 3'-end by using terminal deoxynucleotidyl transferase and labeling reagent (Affymetrix) according to the manufacturer's instructions. The reaction mixtures were incubated at 37°C for 120 min followed by heat inactivation at 95°C for 15 min. Prehybridization of each array was achieved by the addition of 80 μL of prehybridization buffer (10 mM Tris, pH 7.8, 0.01% Tween 20), followed by 15 min of rotation (60 rpm) at 49°C in a Gene Chip hybridization oven 640 (Affymetrix). Labeled samples were added to 72 μL of hybridization master mix (3 M tetra methyl ammonium chloride, 10 mM Tris pH 7.8, 0.01% Tween 20, 500 mg/μL acetylated BSA, 100 mg/mL herring sperm DNA) to a final volume of 98.9 mL. This hybridized cocktail was denatured at 95°C for 5 min and equilibrated at 49°C for 5 min. Prehybridization buffer was removed from the arrays and replaced with 80 mL of hybridized cocktail; then, the arrays were subjected to rotation (60 rpm) for 16 h at 49°C. Arrays were washed and stained by using the "Mini_DNA array_WS5_450" protocol of Fluidic Station 450 and then scanned on a GeneChip scanner

Table 4. PCR conditions

	Amplified region	Length (bp)	Forward Primer (5'-3')	Reverse Primer (5'-3')
PCR condition 1	SCN1A exon3	361	ATC AGT ATA ACG CCA GCA GG	TTG GCT AAG CTG CAG TTT G
98°(10sec)-58°	SCN1A exon4	371	CAT AAG CAC TGA TGG AAA ACC	CTC TTT GTG TTA GGA AGC TGA A
KOD FX (Toyobo)	SCN1A exon5	361	AAA CTC CAA GGC TGA TAA AG	TTC GGT AAT TCC AGG TAA GA
	SCN1A exon12	296	ATG CAC TAT TCC CAA CTC ACA	CCA TTT GGT TGT TTG CTT GTC
	SCN1A exon17	351	TAA ACA AGC TGC ACT CCA AA	GGG TTA GCA CAG ACA ATG AT
	GABRA1 exon10	375	TTC AAG TAG GCT GTC CCA T	CAT ACT CCA TCC AAT ACC ACA
	GABRG2 exon2	310	TGG TCT GTG GAT AAA AGT CAA C	TTG CTC TTG AAC TAC ACT GAT G
	CACNB4 exon3	286	TGA GCA ACG ACC CAC TTT	CAT GGA GTG TGT GCA TCA
	CACNB4 exon13	395	GTC TCC ATC CTA ATC AAG TTC C	GCC CAT ATA GCA CCT CAA AT
	CLCN2 exon5	277	TCT TCC ACC AGG AGG GAC T	CCT CAG GCT GTC GGT ATG TT
	CLCN2 exon19	219	GGT TAA TGA CGT GGT CTC A	CTT ACC TTT CCT GGT CCT CTC
	KCNQ2 exon2	247	AAC AGG AAC GGA AGA CAG AC	TCC TGA GTT CTG GAG ACA CA
	KCNQ2 exon8	318	TGA AAC AGT TGC TTG GTG G	ACA GAG GGT AAA CTG AGG CTG
	KCNQ3 exon5	285	CCT CCA CCT CTC TCT GAC TCT T	GCT CCA GCA TCC ACT CAA C
	CACNA1A exon3	274	CAC CAA CCA AAA GCC TCG TA	GCT GGT TCC CTG ATC TTG TC
	CACNA1A exon33	219	TGA ACC AGG CTC CTC TGA C	CAA GGG AGG AAG ACA GTG C
	CACNA1A exon36	233	GGA GAG GTG AGT ATT GTG GCT	CAC TCA TTC ATT CCC TCG GT
	CACNA1H exon4	287	TGA GCT GAG CTG TTC CAC	CCT CTA GAA CAC ACC TCC CAT
PCR condition 2	SCN1A exon1	574	AGT AGG CAA TTA GCA GCA A	CCT GTA TCA GTA AAG CAC AAG A
98°C (10sec)-58°C	SCN1A exon2	600	AGA TGG TAG AGG TAA ACA TGG T	GAA GAG ATC AGT GA CAG TTT G
KOD FX (Toyobo)	SCN1A exon6	589	AGC CCC TCA AGT ATT TAT CCT	CTT CCA TTA CTC TAT CTC GAC A
	SCN1A exon7	773	ATA GTG CTG CAA TGA ACA TAC G	GCG AGA GGA CCA GAA AAT ATA C
	SCN1A exon8-exon9	1085	ACG ATA AAA GGT CAG TGC CA	AAA GGC AGC AGA ACG ACT
	SCN1A exon10	478	TAA CTA TGT GAA GAA GGG ATG G	CAG TCT CTT CAG GTG CTA TGT T
	SCN1A exon11	700	ATC ATT CTC AAG GTT GCC GT	AGG TTG CAA TCT ATC TCC TCA T
	SCN1A exon13	543	TGA AGG ATG GTT GAA AGA CTG	TGT TAC CTG GGC TCT ATG TGT
	SCN1A exon14	434	TGC AAG AAC CCT GAT TGT TAG	TAT CTA CTT CGC GTT TCC ACA
	SCN1A exon15	526	GTG CCA TGC TGG TGT ATT TC	CGG TTA GGG CAG ATC AGA TAT T
	SCN1A exon16	763	GCC CAG CAT GAG AGT ATA TTG	TGC TCT TCC CTA CAT TGG TG
	SCN1A exon20	807	AAC TCC CAA GAT GGA TTA GG	CAC AGT AGA AAC TTC CTG CTT
	SCN1A exon21	518	CAT CTG GGC TCA TAA ACT TG	AGA CCA GAG ATT ACT AGG GGA A
	SCN1A exon24	412	GTG AGA CAA GCA TGC AAG TT	TGA GAT TTG GGG TGT TTG
	SCN1A exon25	482	TTG CTG GGA TGA TCT TGA AT	TAC AAA AAT CAG GGC CAA TG
	SCN1B exon3	416	AGC CCA TCT GTG TGC CAT	TGT CCC TCC ATC TGG CTC T
	CHRNA7 exon10	599	TCA GGG CTG CTC TTA ACG	CCT TGC CCA TCT GTG AGT
	CHRNB2 exon5	1118	GGT TGA TGG GTA AGG AGG AAG	TAT AGA ACG CCG CTG CTT T
	GABRG2 exon4	703	TGG GTG AGA CAG TAA CCT CC	GCA AAG CGT AGG TGC AAA G
	KCNQ2 exon1	748	CGC TGT ACA ATC GCT CTC C	CTC CCC TCT CCT TCG ACT
	CACNA1H exon7	538	CCC TGA CCC TGA TTG TAC CT	TGA TGT CCA CCC AGC CTT
	CACNA1H exon9	906	CGC TCA CTC ACT GCC ACT T	GAA TGA CAC ACC GGA GAC C
	CACNA1H exon10	632	GCT CCT GTG TGT GAG GGT TC	CCT ACC ATC AGG TCA GGC A
	CACNA1H exon23	445	CTG CTG CGC CTT CTT CAT	TCA CTG GCG AGG GTT AGA
PCR condition 3	SCN1A exon18-exon19	1941	AAG CTA CCT TGA ACA GAG ACA	TAG CAA GAC AGA GAT GAG CAT A
98°C (10sec)-58°C	SCN1A exon22-exon23	2030	CTC ATT TGG CAG AGA AAA CAC	GGG AGG ATA TAT CAA ATG GC
KOD FX (Toyobo)				
PCR condition 4	CHRNA4 exon5	1696	ACC AGG AAG AAA GGG CGT	AAT GCC TCT GTG TGT GGA C
98°C (10sec)-68°C	GABRD exon5-exon6	1024	GTC TGA GAA GTA GCT GGG GCT	TTC CTA GGC AGC AGC GT
KOD FX (Toyobo)	CACNA1H exon11	586	CCA AAC CTG CTC CCA GAT	TGC AGA GAG TGG AGC TTC
PCR condition 5	SCN1A exon26	3387	CCT TCT CCC CCA ATT TGT AA	CGC ATG ATT TCT TCA CTG GT
94°C (15sec)-58°C				
KOD plus (Toyobo)				

(GCS 3000). The data were analyzed by using the Gene Chip Operating Software to obtain signal intensity data and by using the GeneChip Sequence Analysis Software (ver. 4.0) to generate sequence base calls. We used the default set points, with the exception of a quality score threshold of "0" and a genome model of "0".

RESULTS

The *EpiGene* identified 48 *SCN1A* mutations in DNA samples from patients with SMEI, and 44 of these mutations were consistent with mutations identified by using capillary sequencing (Table 1). Heterozygosity was identified in 92% of all cases. No *SCN1A* mutations were found in 125 control samples from healthy volunteers without epilepsy. The call rate was 95%; overall accuracy was >99%; and the false-positive rate was 1.1%. Two patients (S41 and S73) had two mutations, and it is unknown which of the two mutations is related to epilepsy. Our results indicate that *SCN1A* mutations can be correctly identified by using the *EpiGene*. Analyses of other genes tiled on the *EpiGene* are currently ongoing, and the results will be reported separately.

DISCUSSION

SCN1A mutations are found in approximately 80% of SMEI patients. However, several different missense mutations in *SCN1A* have been identified in epileptic patients with benign phenotypes (febrile seizures, SMEI borderlands). Several mutations of *SCN1A* show good response to AED treatment, even if the mutation was identified in SMEI (Kaneko et al., 2008). In such cases, the *EpiGene* will be applicable for screening purposes. Resequencing arrays are new tools for the identification of mutation sites in patients with epilepsy. For high-throughput detection of epilepsy-related mutations, we generated a custom resequencing array for detection of missense and nonsense mutations of *SCN1A* in SMEI patients, and the data generated by using this array were suitable for use in clinical settings. However, the *EpiGene* has limitations; for example, deletion, insertion, and frame-shift mutations are difficult to detect. Although a capillary sequencer can detect approximately 100% of mutations, the *EpiGene* can detect only about 90% of mutations. Because epilepsy is a syndromic disease that includes many phenotypes, numerous genes may contribute to the occurrence of epilepsy. Thus, the *EpiGene* is a powerful tool because it can simultaneously identify many genes in a patient. At present, costs for identification of mutations may be

the same. Currently, we are testing the performance of a new version of *EpiGene* that contains 35 genes, including genes responsible for the side effects of AEDs; this approach will significantly decrease the net cost to perform individualized medicine for epilepsy. The same preparation time is required for the *EpiGene* and capillary sequencer. However the *EpiGene* can simultaneously identify 14 kinds of gene's mutations, while the capillary sequencing approach requires 14 separate assays for a patient with unknown gene mutation. Recently, a next-generation sequencer became available. Although this next-generation sequencer can accurately detect mutations, its cost is too high for widespread clinical use. Our results indicate that the *EpiGene* chip is useful in clinical settings at least for screening purposes, because it can simultaneously and efficiently detect several gene mutations in a patient.

Frontal lobe epilepsy usually responds to carbamazepine, but frontal lobe epilepsy with an S284L mutation in *CHRNA4* responds to zonisamide but not carbamazepine; thus, the drug response is at least partly dependent on the kind of mutations (Zhu et al., 2008). Blumenfeld et al. (2008) reported that early treatment (before onset of epilepsy) of Wistar albino Glaxo rats of Rijswijk (WAG/Rij), a genetic animal model of absence epilepsy, with ethosuximide (300 mg/kg/day), resulted in a favorable outcome. Conceivably, it is possible to prevent epilepsy to some extent, by initiating treatment before the onset of epilepsy. For such an early treatment, the early identification of individuals at risk is also crucial. The *EpiGene* is also applicable for this purpose. Thus, we can expect that a curative treatment strategy for some patients with epilepsy may result from the early introduction of treatment based on genetic data generated with this DNA chip.

CONCLUSIONS

In conclusion, we developed a custom resequencing array, *EpiGene*, that can be used to detect epilepsy-related mutations. This array can be used to detect epilepsy-related mutations at a high rate. Our results indicate that this array could be used in clinical settings.

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REFERENCES

Blumenfeld H., Klein J.P., Schridde U., Vestal M., Rice T., Khera D.S. et al.: *Early treatment suppresses the development of spike-wave epilepsy in a rat model.* Epilepsia, 2008, 49: 400–409.

Claes L., Del-Favero J., Ceulemans B., Lagae L., Van Broeckhoven C., De Jonghe P.: *De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy.* Am. J. Hum. Genet., 2001, 68: 1327–1332.

Claes L.R., Ceulemans B., Audenaert D., Deprez L., Jansen A., Hasaerts D. et al.: *De novo KCNQ2 mutations in patients with benign neonatal seizures.* Neurology, 2004, 63: 2155–2158.

Dravet C., Bureau M., Guerrini R., Giraud N., Roger J.: *Severe myoclonic epilepsy in infants.* In: J. Roger, M. Bureau, C. Dravet, F.E. Dreifuss, A. Perret, P. Wolf (Eds), *Epileptic syndromes in infancy, childhood and adolescence. 2nd ed.*, John Libbey & Company Ltd, London 1992, 75–88.

Fujiwara T., Sugawara T., Mazaki-Miyazaki E., Takahashi Y., Fukushima K., Watanabe M. et al.: *Mutations of sodium channel alpha subunit type 1 (SCN1A) in intractable childhood epilepsies with frequent generalized tonic-clonic seizures.* Brain, 2003, 126: 531–546.

Fukuma G., Oguni H., Shirasaka Y., Watanabe K., Miyajima T., Yasumoto S. et al.: *Mutations of neuronal voltage-gated Na+ channel alpha 1 subunit gene SCN1A in core severe myoclonic epilepsy in infancy (SMEI) and in borderline SMEI (SMEB).* Epilepsia, 2004, 45: 140–148.

Gennaro E., Veggiotti P., Malacarne M., Madia F., Cecconi M., Cardinali S. et al.: *Familial severe myoclonic epilepsy of infancy: truncation of Nav1.1 and genetic heterogeneity.* Epileptic Disord., 2003, 5: 21–25.

Hacia J.G.: *Resequencing and mutational analysis using oligonucleotide microarrays.* Nat. Genet., 1999, 21: 42–47.

Kaneko S,, Yoshida S., Kanai K., Yasui-Furukori N., Iwasa H.: *Development of individualized medicine for epilepsy based on genetic information.* Expert Rev. Clin. Pharmacol., 2008, 1: 661–681.

Kearney J.A., Wiste A.K., Stephani U., Trudeau M.M., Siegel A., Ramachandran Nair R. et al.: *Recurrent de novo mutations of SCN1A in severe myoclonic epilepsy of infancy.* Pediatr. Neurol., 2006, 34: 116–120.

Kwan P., Brodie M.J.: *Early identification of refractory epilepsy.* Engl. J. Med., 2000, 342: 314–319.

Madia F., Striano P., Gennaro E., Malacarne M., Paravidino R., Biancheri R. et al.: *Cryptic chromosome deletions involving SCN1A in severe myoclonic epilepsy of infancy.* Neurology, 2006, 10: 1230–1235.

Mulley J.C., Nelson P., Guerrero S., Dibbens L., Iona X., McMahon J.M. et al.: *A new molecular mechanism for severe myoclonic epilepsy of infancy: exonic deletions in SCN1A.* Neurology, 2006, 67: 1094–1095.

Nabbout R., Gennaro E., Dalla Bernardina B., Dulac O., Madia F., Bertini E. et al.: *Spectrum of SCN1A mutations in severe myoclonic epilepsy of infancy.* Neurology, 2003, 24: 1961–1967.

Nasir M., Olga V., Anthony M., Munir.: *Exploring the genomic basis of pharmacoresistance in epilepsy: an integrative analysis of large-scale gene expression profiling studies on brain tissue from epilepsy surgery.* Hum. Mol. Genet., 2011, 20: 4381–4394.

Ohmori I., Ouchida M., Ohtsuka Y., Oka E., Shimizu K.: *Significant correlation of the SCN1A mutations and severe myoclonic epilepsy in infancy.* Biochem. Biophys. Res. Commun., 2002, 295: 17–23.

Ottman R., Hirose S., Jain S., Lerche H., Lopes-Cendes I., Noebels J.L. et al.: *Genetic testing in the epilepsies-report of the ILAE Genetics Commission.* Epilepsia, 2010, 51: 655–670.

Reid C.A., Berkovic S.F., Petrou S.: *Mechanisms of human inherited epilepsies.* Prog. Neurobiol., 2009, 87: 41–57.

Saitoh M., Shinohara M., Hoshino H., Kubota M., Amemiya K., Takanashi J.L. et al.: *Mutations of the SCN1A gene in acute encephalopathy.* Epilepsia, 2012, 53: 558–564.

Shi L., Reid L.H., Jones W.D., Shippy R., Warrington J.A., Baker S.C. et al.: *The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements.* Nat. Biotechnol., 2006, 24: 1151–1161.

Steinlein O.K., Magnusson A., Stoodt J., Bertrand S., Weiland S., Berkovic S.F. et al.: *An Insertion Mutation of the CHRNA4 gene in a family with autosomal dominant nocturnal frontal lobe epilepsy.* Human. Mol. Genet., 1997, 6: 943–947.

Steinlein O.K., Mulley J.C., Propping P., Wallace R.H., Phillips H.A., Sutherland G.R. et al.: *A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy.* Nat. Genet., 1995, 11: 201–203.

Sugawara T., Tsurubuchi Y., Agarwala K.L., Ito M., Fukuma G., Mazaki-Miyazaki E. et al.: *A missense mutation of the Na+ channel alpha II subunit gene Na(v)1.2 in a patient*

with febrile and afebrile seizures causes channel dysfunction. Proc. Natl. Acad. Sci. USA, 2001, 98: 6384–6389.

Suls A., Claeys K.G., Goossens D., Harding B., Van Luijk R., Scheers S. et al.: *Microdeletion involving the SCN1A gene may be common in SCN1A-mutation-negative SMEI patients.* Hum. Mutat., 2006, 27: 914–920.

Wang J.W., Kurahashi H., Ishii A., Kojima T., Ohfu M., Inoue T. et al.: *Microchromosomal deletions involving SCN1A and adjacent genes in severe myoclonic epilepsy in infancy.* Epilepsia, 2008, 49: 1528–1534.

Zhu G., Okada M., Yoshida S., Ueno S., Mori F., Takahara T. et al.: *Rats harboring S284L Chrna4 mutation show attenuation of synaptic and extrasynaptic GABAergic transmission and exhibit the nocturnal frontal lobe epilepsy phenotype.* J. Neurosci., 2008, 28: 12465–12476.