Poster Presentation: ACMG 2007

Analysis of SCN1A gene for pathogenic mutations in Severe myoclonic epilepsy of infancy (SMEI) by DHPLC, SURVEYOR® Nuclease and DNA sequencing: A sensitive efficient clinical approach

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ABSTRACT

Severe myoclonic epilepsy of infancy (SMEI) has been characterized by generalized tonic, clonic and tonic-clonic seizures that are initially induced by fever and begin during the first year of life. Later in life, patients with SMEI have afebrile seizures, including myoclonic, tonic-clonic, absence, and simple and complex partial seizures. Early psychomotor and speech development is normal, but development is delayed. In general SMEI is very resistant to all forms of pharmacotherapy.

Mutations of the alpha1 subunit sodium channel gene (SCN1A) cause SMEI. Mutations of SCN1A have been found in 40 to 100% of SMEI patients, depending on population, and are de novo in the majority of individuals. Reports have indicated SCN1A mosaic mutation is correlated with the milder phenotype, whereas the full heterozygous mutation caused SMEI. The possibility of mosaic mutations must, therefore, also be taken into account for genetic counseling and determining the recurrence risk in patients with SMEI. Heteroduplex based scanning methodologies, DHPLC and SURVEYOR Nuclease combined with selective DNA sequencing provide a sensitive approach to detect even low levels of mosaicism which account for milder phenotypes of SMEI.

Samples have been analyzed with our multipronged approach in which a total of 29 amplicons covering the coding and splice junction regions of the SCN1A gene. They are PCR-amplified with a high–fidelity DNA polymerase and analyzed by DHPLC under fully optimized conditions for mutation screening. Scanning results are further confirmed with SURVEYOR Nuclease and DNA sequencing. This strategy efficiently detects point mutations, deletions, insertions and splice donor site mutations in SCN1A gene with a detection limit of 0.5 % heterozygous. Collection and re-amplification of low degree heteroduplex peak-fractions allows sequence analysis of low level mutations. This method provides a sensitive assay with an increased detection rate of low levels of mutations in a rapid, cost-effective manner.

INTRODUCTION

SMEI has been recently recognized among childhood epilepsy populations with the discovery of mutations in the neuronal SCN1A gene. SMEI typically presents with recurrent febrile hemiclonic or generalized status epilepticus at around age 6 months. Myoclonic seizures appear between ages 1 and 4. SMEI is associated with a significant mortality in childhood. SCN1A-sodium channel neuronal voltage–gated alpha subunit is located on chromosome 2q. Mutations have been located within the transmembrane segments DII S4 and DIV S4, which are voltage sensors of the channel, the linker between DII and DIII and in the DI S2-S3 loop, DIII S5 and DIV S4, see Figure 1.

We present data from analysis of an example group with a sensitive, cost effective and less labor intensive approach. All 26 exons and exon-intron boundaries of the SCN1A gene were amplified with universal PCR conditions and scanned with rapid-sensitive mutation detection using DHPLC and heteroduplex cleavage fragment analysis (SURVEYOR) on the WAVE® HS System. Variants were analyzed with bidirectional, double strand sequencing.

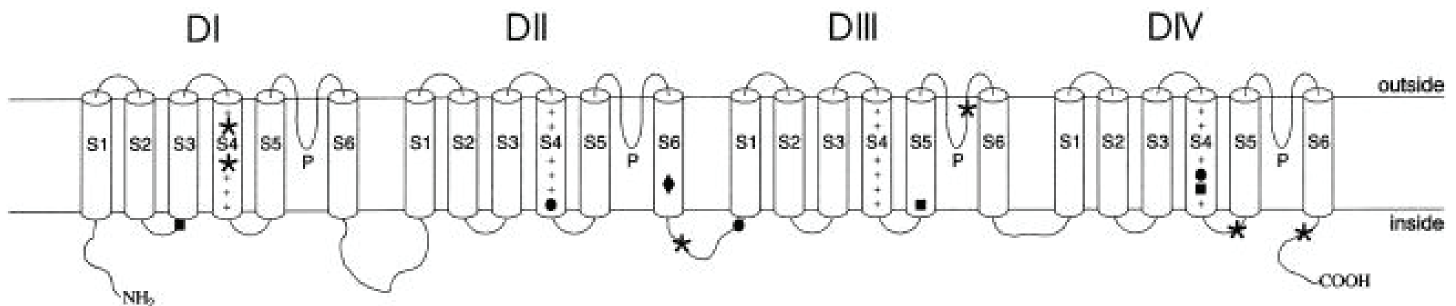

Figure 1. Organization of the SCN1A product complex. The neuronal voltage-gated sodium-channel α-subunit SCN1A is a monomer and consists of four homologous domains (DI–DIV). Each domain has six transmembrane segments (S1–S6). S4 has several positively charged amino acids and represents the voltage sensor. P = the pore loop, which delineates the pore of the channel, see reference 2.

METHODS AND RESULTS

After PCR amplification of all amplicons, an aliquot was analyzed by DHPLC on the WAVE HS System. Each amplicon used specific conditions designed to scan the length of the PCR product. Thus, comparison to the wild-type showed any variation from the wild-type, see Figure 2.

= Wild Type = Variation

Figure 2. SCN1A DHPLC analysis. Samples were screened for heterozygous mutations within amplicon 26C using PCR and subsequent analysis with the WAVE HS System. The temperatures from upper left to right and lower left to right were 54.7, 55.9, 56.9 and 57.3°C. The sample clearly showed a variation from the wild type.

Heteroduplex cleavage fragment analysis was performed with SURVEYOR Nuclease and the WAVE HS System. SURVEYOR cuts amplicons where there is a heteroplasmic variation. The presence and sizes of these fragments were viewed on the WAVE, see Figure 3.

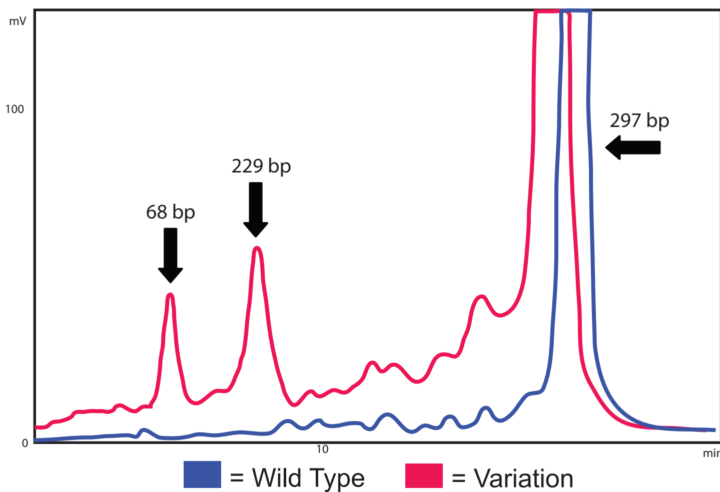

Figure 3. SCN1A SURVEYOR Analysis. All representatives were screened for mutations following PCR by using SURVEYOR heteroduplex scanning and the WAVE HS System for detection. The sample showed a variant that split the amplicon from 297bp into 68 and 229bp fragments.

Finally, selected amplicons were analyzed by bi-directional, double strand sequencing on an ABI 3100 automated sequencer (Applied Biosystems), see Figure 4. Variant identities were determined and labeled as mutational or as a common single nucleotide polymorphism (SNP), see Table 1.

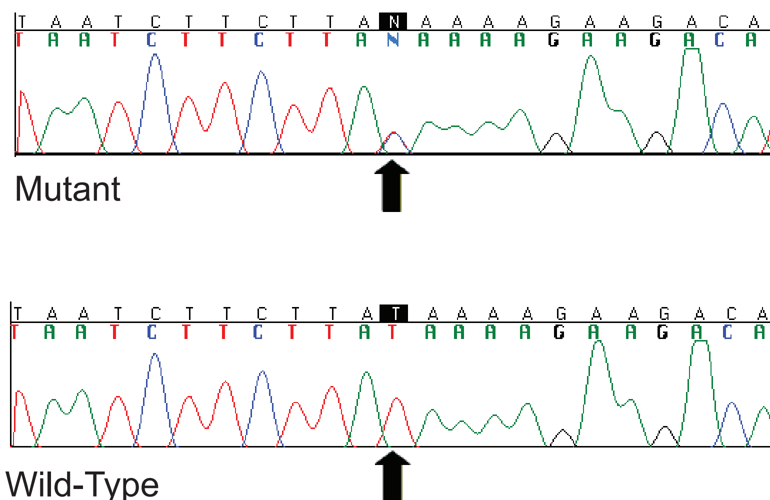

Figure 4. SCN1A Sequencing. Samples that were found to be positive via DHPLC and SURVEYOR heteroduplex scanning were directly sequenced. The sample variant was shown to be the Ile>1944>Thr SNP caused by aTa>aCa.

Table 1. Complete group results of somatic screening of the SCN1A gene

Amplicon/ Exon	DNA Variant	Genetic Annotation	Amino Variant	Genewindow Notation		
				dbSNP ID	Nt	Chr
1	A>T	-82	n/a	rs566839	17139632	166638460
2	aaT>aaC	Ex2+81	Asn115Asn	n/a	n/a	n/a
2	T>C	IVS2+66	n/a	rs8191987	17124432	166623260
5	G>T	IVS4-106	n/a	rs3812719	17118977	166617805
5	G>A	IVS4-91	n/a	rs3812718	17118962	166617790
7	C>T	IVS6-21	n/a	rs994399	17114898	166613726
7	T>C	IVS7+21	n/a	rs1542484	17114793	166613621
8	C>T	IVS7-68	n/a	rs1461193	17113764	166612592
8	C>T	IVS8+112	n/a	rs11690959	17113443	166612271
9	gtA>gtG	Ex9-42	Val404Val	rs7580482	17112863	166611691
9	G>A	IVS9+52	n/a	rs6432861	17112646	166611474
10	delA	IVS9-161	n/a	rs5836074	17111416	166610244
11	T>G	IVS10-47	n/a	rs6753355	17110024	166608852
13	insT	IVS12-25	n/a	rs11397312	17107421-17107422	166606249-166606250
13	gtT>gtC	Ex13+116	Val753Val	rs6432860	17107282	166606110
14	G>A	IVS13-72	n/a	rs490317	17105596	166604424
14	A>C	IVS13-37	n/a	rs2126152	17105561	166604389
14	delA	IVS14+3	n/a	rs35541995	17105348	166604176
14	delA	IVS14+4	n/a	rs35860073	17105347	166604175
14	delA	IVS14+5	n/a	rs34347391	17105346	166604174
16	C>T	IVS15-41	n/a	rs7601520	17102499	166601327
16	Gca>Aca	Ex16-231	Ala1056Thr	rs2298771	17102206	166601034
18	G>A	IVS18-67	n/a	n/a	17079893	166578721
18	C>G	IVS18+55	n/a	n/a	17079617	166578445
21	T>C	IVS20-69	n/a	rs1350798	17068750	166567578
23	insA	IVS22-123	n/a	rs35180275	17064225	166563053
26	aTa>aCa	Ex26-199	Ile1944Thr	rs35735053	17057339	166556167

CONCLUSIONS

- Validation of this mutation screening approach to regions that harbor the most prevalent pathogenic mutations was confirmed by the successful detection of any and all reference mutations in test examples.
- Amplicons were designed and optimized for both accuracy and efficiency in the mutation screening process.
- PCR primers and conditions were designed and optimized to facilitate the use a single thermal cycling condition, while improving specificity and yield of all products for analysis. This heightened quality and minimized the resources required.
- DHPLC analysis conditions were optimized for mutation detection in each amplicon with use of a minimal range of different temperatures. This minimized process time.
- The SCN1A gene was screened using this multi-pronged approach with independent screening technologies. This ensured discovery of all variants and with greater sensitivity than by sequencing alone.
- We have developed and validated an in-depth screening methodology for mutational analysis of the SCN1A gene for mutations. Low levels of heterozygous mutations of <1% are easily detected. The versatility of the WAVE HS System, coupled with SURVEYOR Nuclease and sequencing, have been successfully applied in these examples with SCN1A variations for a cost-effective and thorough analysis of somatic mutations.
- This comprehensive analysis for testing can be completed within 5 weeks and is very competitive in both speed and cost.

REFERENCES

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3. National Cancer Institute, Core Genotyping Facility: genewindow.nci.nih.gov

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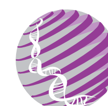

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