

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.

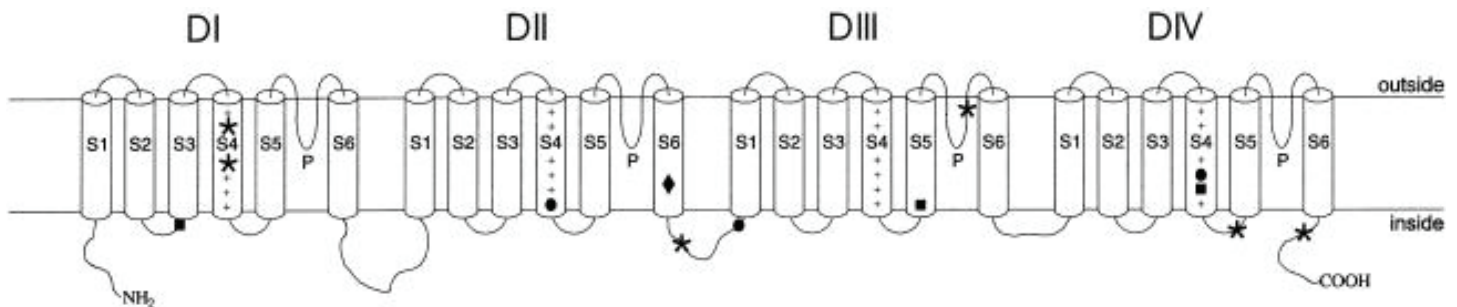


Figure 1. Organization of the SCN1A product complex. The neuronal voltage-gated sodium-channel α -subunit SCN1A is a monomer and consists of four homologous domains (DI–DIV). Each domain has six transmembrane segments (S1–S6). S4 has several positively charged amino acids and represents the voltage sensor.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

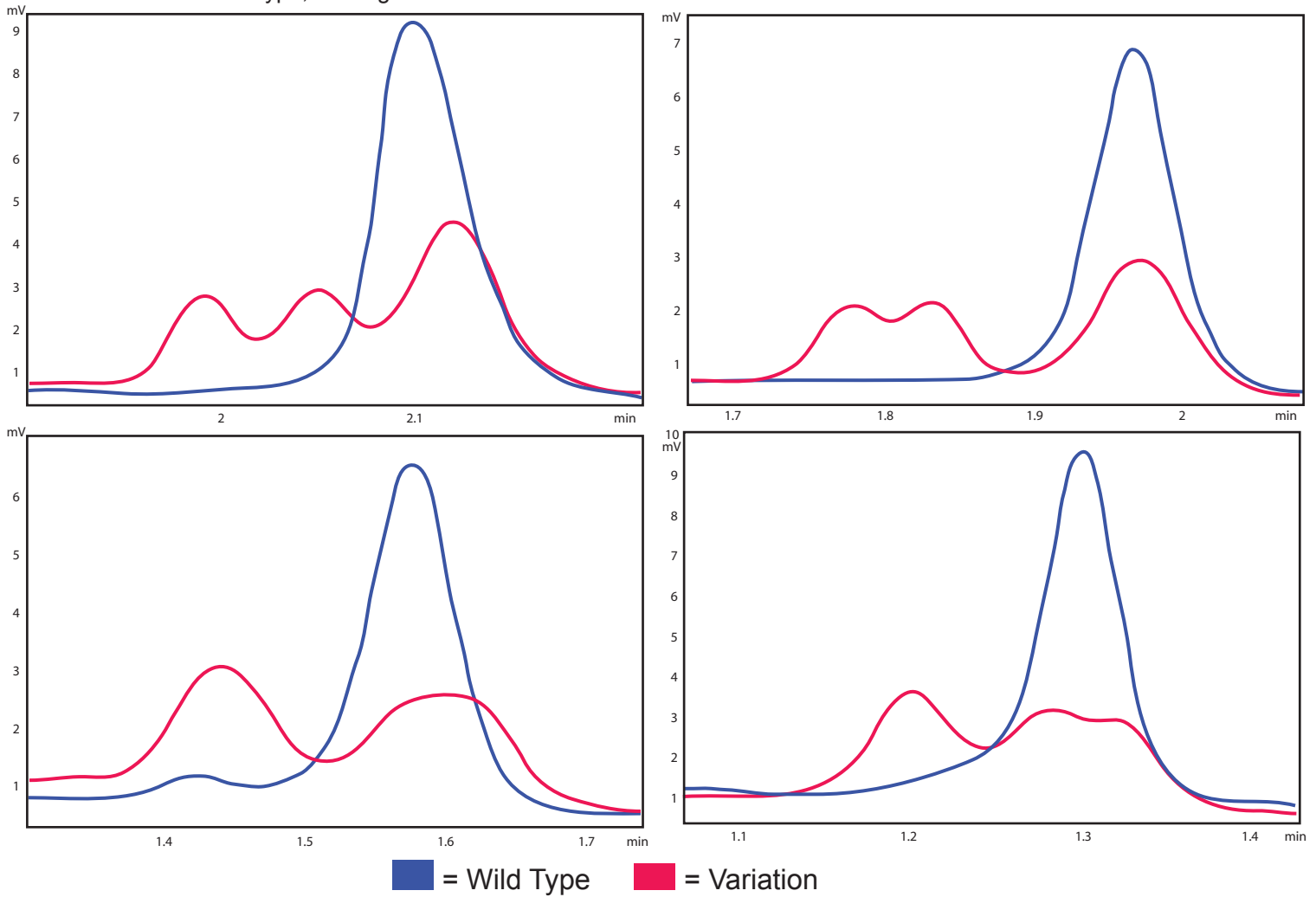


Figure 2. SCN1A DHPLC analysis. Samples were screened for heterozygous mutations within amplicon 26C using PCR and subsequent analysis with the WAVE HS System. The temperatures from upper left to right and lower left to right were 54.7, 55.9, 56.9 and 57.3°C. The sample clearly showed a variation from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

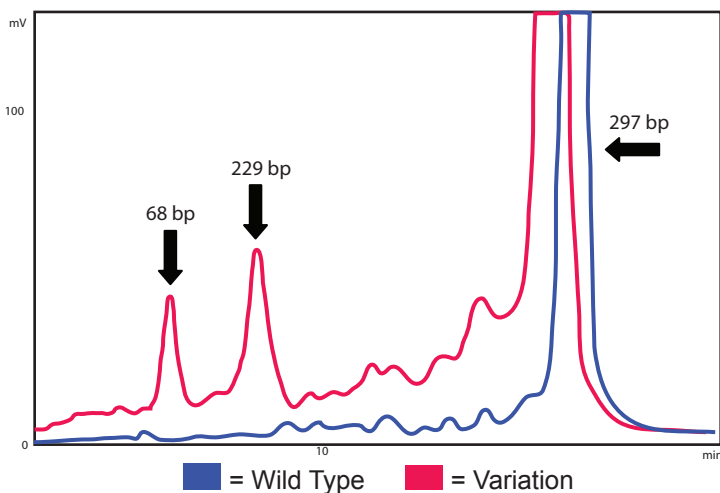


Figure 3. SCN1A SURVEYOR Analysis. All representatives were screened for mutations following PCR by using SURVEYOR heteroduplex scanning and the WAVE HS System for detection. The sample showed a variant that split the amplicon from 297bp into 68 and 229bp fragments.

