

SEARCHING FOR THE MESIAL TEMPORAL LOBE EPILEPSY GENE: VALIDATING CANDIDATE VARIANTS IDENTIFIED BY NEXT GENERATION SEQUENCING

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Abstract

A family affected by mesial temporal lobe epilepsy was studied. Our group studied possible candidate variants through Sanger sequencing methods in order to compare with Next-Generation Sequencing. None of the mutations was confirmed, characterizing the methods' low efficacy and need of further studies about such.

Key words: epilepsy, next-generation sequencing, genetics.

Introduction

Mesial temporal lobe epilepsy is the most common form of human epilepsy, and familial forms have been reported. A candidate locus on chromosome 18p11.31 was identified, through genome-wide linkage study in a large family with autosomal dominant transmission. In order to search for the causative mutation, genes localized in the 18p11.31 locus were amplified by long range PCR and sequenced by next-generation sequencing (NGS) in an ABI Solid SystemTM. Bioinformatics analysis revealed 32 deleterious candidate variants localized in 11 genes. The objectives were to validate deleterious candidate variants identified by NGS in a family segregating MTLE linked to ch 18p11.31. We studied a total of 28 family members, 14 patients. Genomic DNA was isolated from lymphocytes of fresh blood by standard methods. Genomic regions containing the variants were amplified by polymerase chain reaction. Amplicons were submitted to capillary electrophoresis in a sequencer ABI 3500XL genetic analysis. Analysis involved Chromas software.

Results and Discussion

All affected individuals were genotyped for 17 variations located at the following genes L3MBTL4 (exon 15), EPB41L3 (exons 13 e 23), LAMA (exons 29, 32, 41, 43, 62), LRRC30 (exon 1) and ARHGAP28 (exon 12). From the candidate SNPs found by NGS, 13 were not validated by Sanger sequencing.

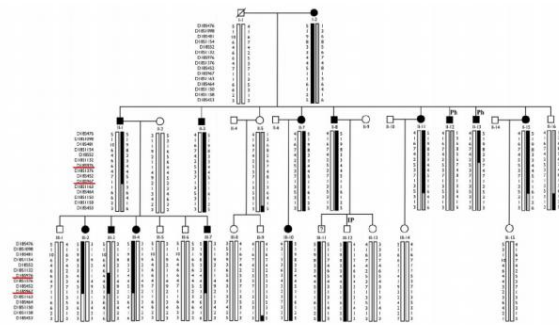


Image 1. Pedigree of the family studied. The haplotype inherited by affected individuals is represented by blackened vertical bars. Microsatellite markers in red show the minimum candidate region that contains the MTLE gene.

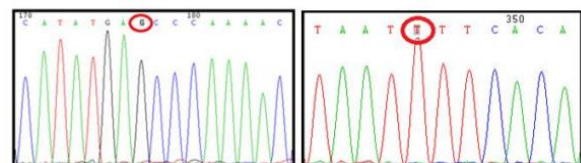


Image 2. On the left, Sanger sequencing of LAMA1 gene (exon 62). The red circle shows "wild type" allele G, instead of mutant allele C. On the right, the gene EPB41L3 (exon 23).

Conclusions

None of nucleotide changes identified by NGS on ch 18p11.31 candidate locus were not validated by Sanger sequencing. Therefore, more studies are necessary to define the major gene predisposing to MLTE with hippocampal atrophy.

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