Offering hope and changing lives through research

INGEMM Genetic Test Application
Technical Report
European delegation of Dravet syndrome Foundation
TECHNICAL REPORT

Introduction

The main objective of this project is to provide a genetic diagnosis for those patients who suffer from Dravet Syndrome (DS), a rare and severe form of epilepsy, usually occurring in the first year of life. In addition, genetic diagnosis for other types of epilepsies will also be carried out, including: febrile seizures, febrile seizures plus, generalized epilepsy with febrile seizures plus, severe myoclonic epilepsy borderline in infancy and intractable childhood epilepsy.

The selection process for patient inclusion in the DS genetic diagnosis project will be carried out by the INGEMM. During the development of the project, further clinical information or a medical record updating should be provided by the requesting doctor in order to exclusively analyse DS patients.

Firstly, a mutation screening of the genes linked to date to DS will be performed. Point mutations and partial or whole gene deletions/duplications of the \textit{SCN1A} gene are responsible for 70-80\% of the patients with DS. A small proportion of DS patients (5\%), females in majority, carry \textit{PCDH19} genetic alterations, including point mutations and partial or whole gene deletions of this gene. Rare point mutations have also been identified in the \textit{GABRG2}, \textit{SCN2A} and \textit{SCN1B} genes. Recently, a modifying effect of \textit{SCN9A} mutations in combination with \textit{SCN1A} mutations has been postulated as a genetic risk factor for Dravet syndrome.

Despite these advances, the etiology of about 20\% of DS patients remains unknown, and additional genes are likely to be implicated, thus, the second main objective of this project is to identify new genes involved in DS.

Project: phases and methodology

The first step is to obtain DNA from a patient blood sample (or other tissue). Afterwards, different phases will be initiated:

\textbf{Phase 1: Mutation screening of the \textit{SCN1A} gene in patients with DS and its related spectrum disorders}

First of all, mutation screening of the \textit{SCN1A} gene, responsible for the majority of cases of DS, will be performed.

\textit{SCN1A} point mutations detection will be carried through amplification by PCR of its coding and intron-exon boundaries, following direct sequencing of the PCR products.

Detection of whole or partial \textit{SCN1A} deletions and duplications will be carried out using the MLPA (Multiplex Ligation dependent Probe Amplification) assay.
Finally, a genetic report will be submitted to the requesting doctor. Estimated delivery time of the report is 4 months.

**Phase 2: Mutation screening of the PCDH19 gene in SCN1A-negative DS females.**

Mutation screening of the PCDH19 gene will only be performed in DS females since males with DS and a PCDH19 mutation are extremely rare and difficult to detect with the routine techniques employed in the genetic diagnostics laboratories.

PCDH19 point mutations detection will be carried out through amplification by PCR of its coding and intron-exon boundaries, following direct sequencing of the PCR products.

Due to the absence of a commercial MLPA for the detection of whole or partial PCDH19 deletions, a self-designed MLPA assay for this gene will be developed.

Finally, a genetic report will be submitted to the requesting doctor. Estimated delivery time of the report is yet to be determined.

**Phase 3: Mutation screening of the GABRG2, SCN2A and SCN1B genes in DS patients with unknown molecular defect.**

During this phase a mutation screening of the genes rarely associated with DS will be performed: GABRG2, SCN2A and SCN1B.

Detection of GABRG2, SCN2A and SCN1B point mutations will be carried out through amplification by PCR of their coding and intron-exon boundaries of these genes, following a variation screening using HRM (High Resolution Melting) and subsequent sequencing of any identified variant.

Finally, a genetic report will be submitted to the requesting doctor. Estimated delivery time of the report is yet to be determined.

**Phase 4: Mutation screening of the SCN9A gene in SCN1A-positive DS families.**

In those families with members which present the same SCN1A mutation but distinct phenotype, it will be determined if SCN9A variations negatively modify the pathogenic effect of the SCN1A mutation.

Detection of SCN9A point mutations will be carried out through amplification by PCR of their coding and intron-exon boundaries of this gene, following a variation screening using HRM and subsequent sequencing of any identified variant.
Finally, since this is a research phase, a genetic report will be submitted to the requesting doctor only if the obtained results have a significant impact to the health of the patients.

**Phase 5: Screening of novel genetic alterations involved in DS using new technology**

In order to identify novel genetic alterations responsible for the DS phenotype diagnosed to those patients with unknown molecular defect, the newest technology will be employed: exome sequencing and array CGH (Comparative Genomic Hybridization).

Exome sequencing allows the detection of point mutations in the coding regions of the human genome. Up to 85% of the disease causing mutations are located in these regions, thus, this technique may identify new genes involved in the DS.

Array CGH detects CNVs (Copy Number Variations) located all over the human genome. CNVs are responsible of disease in 5-10% of the patients diagnosed with other pathologies distinct from the DS; therefore, this diagnostic tool could discover pathogenic CNVs in genes not yet involved in DS.

Finally, since this is a research phase, a genetic report will be submitted to the requesting doctor only if the obtained results have a significant impact to the health of the patients.