Test Information Sheet

SCN1A

Genomic Diagnostics (GD)

Genomic Diagnostics
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Requirements for Testing:

- Peripheral blood (into EDTA tube) (2x9mL for full screen, 9mL for Predictive test), one buccal swab (supplied by GD) or high quality DNA (A260/280>1.7) (2ug for full screen and 0.5ug for Predictive test). [1]
- Test Request Form must be completed by referring clinician.

  Note: To optimize the accuracy of the personalised management for the patient, please complete entire form to avoid delays.
- Copy of patient's (or guardian) consent for the analysis.
- Completed payment form (for private funded tests only). Testing can only commence once a payment has been made

Description of Analysis

Comprehensive SCN1A gene Analysis:
Full sequence determination in both forward and reverse directions of approximately 8,112 base pairs comprising 26 exons and additionally approximately 1050 adjacent non-coding intronic base pairs. The wild-type SCN1A gene encodes a protein comprised of 2,009 amino acid residues. The non-coding intronic regions of SCN1A that are analysed do not extend more than 20 base pairs proximal to the 5' end and 10 base pairs distal to the 3' end of each exon. The SCN1A comprehensive analysis also includes the detection of large genomic rearrangements (deletions and duplications). The analysis is performed with Multiplex Ligation-dependent Probe Amplification (MLPA) Assay where the amplification products are separated by capillary electrophoresis. The SALSA MLPA kit contains probes for all 26 exons of the SCN1A gene, as well as 14 reference probes for sequences located in other genes. In addition, it contains 9 control fragments generating an amplification product smaller than 120nt: four DNA Quantity fragments. (Q-fragments) at 64-70-76-82nt, three DNA denaturation control fragments (D fragments) at 88-92-96nt, one X-fragment at 100nt and one Y-fragment at 105nt. Apparent SCN1A deletions/duplications of a single/multiple exon(s) are confirmed by an independent MLPA analysis. The Comprehensive SCN1A gene analysis is believed to rule out the majority of genetic abnormalities in this gene. Data on polymorphic variants are available upon request.

Single Site SCN1A gene Analysis (Predictive/Segregation Analysis): DNA sequence analysis (or MLPA analysis) for a specified mutation/genetic variant in SCN1A gene.

Description of Method:

DNA is extracted and purified from white blood cells and from buccal cells isolated from each sample. Aliquots of patient's DNA are each subjected to polymerase chain reaction (PCR) amplification (35 reactions for SCN1A). The amplified products are each directly sequenced in forward and reverse directions using fluorescent dye direct sequencing protocol. Chromatographic tracings of each amplicon are analysed by a proprietary computer-based review followed by visual inspection and confirmation. Genetic variants are detected by comparison with a consensus wild-type sequence constructed for each gene. All potential mutations and genetic variants of unknown clinical significance are independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination as above.
For Single locus specific SCN1A mutation Analysis (Predictive/Segregation Testing), duplicate PCR reactions are performed and the PCR products are analysed by direct DNA sequencing. Controls for normal and mutated sequences (where available) are co-analysed for QA purposes.
Genomic rearrangements are detected by Multiplex Ligation dependent Probe Amplification (MLPA) Assay. The patient's DNA sample is compared with positive and negative controls for single/multiple exon deletions and duplications (where available). Apparent deletions/duplications of a single/multiple exon(s) are confirmed by reanalysing the DNA from the original specimen in duplicate. To avoid the misinterpretation of the MLPA results [due to close proximity of a mutation and/or polymorphisms to the probe ligation site] the apparent single exon deletion's probe annealing site and nearby region (50bp upstream and 50bp downstream from the probe binding site) is always sequenced.

Performance Characteristics:

Analytic specificity: The incidence of a false report of a genetic variant or mutation resulting from technical error is considered negligible because of independent confirmation of all genetic variants (see above). The incidence of a false report of a genetic variant or mutation resulting from errors in specimen handling and tracking is estimated from validation studies to be less than one percent (<1%). Confirmation testing using a second patient's specimen for all mutations identified is recommended.

Analytic sensitivity: Failure to detect a genetic variant or mutation in the analysed DNA regions may result from errors in specimen handling and tracking, amplification and sequencing reactions, or computer-assisted analysis and data review. The rate of such errors is estimated from validation studies to be less than one percent (<1%).

Overall test accuracy: For a patient with at least a 10% probability of a positive test result based on a personal or family history of cancer.

Limitations of method: There may be limited portions of SCN1A gene for which sequence determination can be performed only in the forward or reverse direction. Unequal allele amplification may result from rare polymorphisms under primer annealing sites. This assay will not detect any types of errors in RNA transcript processing. This analysis, however, is believed to rule out the majority of abnormalities in this gene.
Confirmation testing using a second sample for all mutations identified is recommended.

Interpretive Criteria:

"Positive for a deleterious mutation": A positive test result means that the laboratory found a change in SCN1A gene. Depending on the purpose of the test, this result may confirm a diagnosis, indicate that a person is a carrier of a particular genetic mutation, identify an increased risk of developing a disease in the future, or suggest a need for further testing. Because family members have some genetic material in common, a positive test result may also have implications for certain blood relatives of the person undergoing testing.

Positive test result indicates the presence of the mutations (nonsense, insertions, deletions) that prematurely terminate

[1] *For some test requests an alternative source of genetic material can be accepted for testing (please contact Genomic Diagnostics for details)*

("truncate") the protein product of SCN1A. In addition, specific missense mutations and non-coding intervening sequence (IVS) mutations are recognized as deleterious on the basis of data derived from linkage analysis of high risk families, functional assays, biochemical evidence and/or demonstration of abnormal mRNA transcript processing.

"Genetic variant of uncertain significance": A genetic variation (or variant) of unknown significance may or may not be associated with disease. A possible disease variant is suspected to be associated with disease, but this association remains quite uncertain. More information is needed to clarify the significance of such genetic variations. Over time, this information may become available in the literature. Genetic testing of affected individuals within the patient's extended family (known as concordance testing) may also yield this information. If all affected family members harbor the genetic variation in question, it is likely to be associated with the inherited disorder. If some affected family members do not harbor the genetic variation in question, it is less likely to be associated with the inherited disorder. Clarifying the significance of a genetic variation through concordance testing in a family not only benefits this particular family, but also other families who harbor the same genetic variation. Genetic variants of uncertain significance include missense mutations and mutations that occur in analysed intronic regions whose clinical significance has not yet been determined. A genetic variant of uncertain significance in SCN1A is considered to be less likely to be deleterious if it has been observed in one or more individuals with a known deleterious mutation in the same gene. The specific evidence supporting such an interpretation will be summarized for individual variants on each such report.

"No deleterious mutation detected": A negative test result means that the laboratory did not find a dangerous copy of the *SCN1A* gene. This result can indicate that a person is not affected by a particular disorder, is not a carrier of a specific genetic mutation, or does not have an increased risk of developing a disease. It is possible, however, that the test missed a disease-causing genetic alteration because many tests cannot detect all genetic changes that can cause a particular disorder. Further testing may be required to confirm a negative result. Negative test result includes that the non-truncating genetic variants observed at an allele frequency of approximately 1% of a suitable control population (providing that no data suggest clinical significance), as well as all genetic variants for which published data demonstrate absence of substantial clinical significance. Also includes mutations in the protein-coding region that neither alter the amino acid sequence nor are predicted to significantly affect exon splicing, and base pair alterations in non-coding portions of the gene that have been demonstrated to have no deleterious effect on the length or stability of the mRNA transcript. In some cases, a negative result might not give any useful information. This type of result is called uninformative, indeterminate, inconclusive, or ambiguous. Uninformative test results sometimes occur because everyone has common, natural variations in their DNA, called polymorphisms that do not affect health. If a genetic test finds a change in DNA that has not been associated with a disorder in other people, it can be difficult to tell whether it is a natural polymorphism or a disease-causing mutation. An uninformative result cannot confirm or rule out a specific diagnosis, and it cannot indicate whether a person has an increased risk of developing a disorder. In some cases, testing other affected and unaffected family members can help clarify this type of result.

"Specific variant/mutation not identified": Specific and designated deleterious mutations or variants of uncertain clinical significance are not present in the individual being tested.

Change of interpretation and issuance of amended reports: If and whenever there is a change in the clinical interpretation of a specific reported variant, an amended test report will automatically be provided by Genomic Diagnostics.

Description of Nomenclature:

In the Human Genome Variation Society (HGVS) standard nomenclature (http://www.hgvs.org) the nucleotide numbering is in relation to the translation initiation codon, starting with number 1 at the A of the ATG translation initiation codon. The GenBank Accession numbers for SCN1A gene is AB093548.1

Quality Assurance:

All tests are performed according to the AS/ISO15189 standard in a NATA and RCPA accredited laboratory. Accreditation No: 19619.