Ambry Genetics®

SAMPLE REPORT

FINAL REPORT - 8/25/2020

Ordered By
Physician: Unknown, Unknown, MD
Client: Ambry

Additional Authorized Recipient:
Sample GC, CGC

Patient Name: **Patient, Sample**
Accession #: **00-109616**
AP2 Order #: 835488

Birthdate: 01/01/1976
MRN #: N/A
Indication: Diagnostic

Specimen #: N/A
Specimen: Blood EDTA
Gender: M
Collected: 07/30/2020
Received: 07/30/2020

EpilepsyNext®: Analyses of 124 Genes Associated With Epilepsy

RESULTS

Gene	Inheritance	Alteration	Proband
***SCN1A* (NM_001165963)**	**Autosomal dominant**	**Pathogenic Mutation: c.1027G>C (p.G343R)**	**Heterozygous**

SUMMARY

POSITIVE: Pathogenic Mutation Detected

INTERPRETATION

- This individual is is heterozygous for the **c.1027G>C (p.G343R)** pathogenic mutation in the *SCN1A* gene.
- This result is consistent with a diagnosis of *SCN1A*-related seizure disorder.
- The expression and severity of disease for this individual cannot be predicted.
- Genetic testing for pathogenic mutations in family members can be helpful in identifying at-risk individuals.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

No additional pathogenic mutations or gross deletions or duplications were detected. This patient did not opt in to reporting of variants of uncertain significance.

***SCN1A* Additional Information**

Gene Symbol	RefSeq ID	Genomic Coordinates (GRCh37)	Genomic Size (bp)	Total Exons	Coding Exons	Number of Amino Acids
SCN1A	NM_001165963	chr2:166845670-166930149	84480	26	26	2009 aa

VARIANT DETAILS:

- The p.G343R pathogenic mutation (also known as c.1027G>C), located in coding exon 7 of the *SCN1A* gene, results from a G to C substitution at nucleotide position 1027. The glycine at codon 343 is replaced by arginine, an amino acid with dissimilar properties.
- Two different alterations at this same amino acid position, p.G343C and p.G343D, have been reported in individuals with *SCN1A*-related epilepsy (Fujiwara T, 2003).
- Based on internal structural analysis, this variant is anticipated to result in a significant decrease in structural stability (Shen H, 2017; Ambry internal data).
- The p.G343R alteration is predicted to be deleterious by *in silico* analysis.
- Based on the available evidence, the *SCN1A* c.1027G>C (p.G343R) alteration is classified as pathogenic.

GENE INFORMATION:

The *SCN1A* gene is located on chromosome 2q24.3 and encodes the sodium channel protein type 1 subunit alpha. Pathogenic alterations in this gene have been associated with familial hemiplegic migraine, which is inherited in an autosomal dominant fashion, and a spectrum of *SCN1A*-related seizure disorders including generalized epilepsy with febrile seizures plus (GEFS+) and familial febrile seizures, which are inherited in an autosomal dominant fashion, and Dravet syndrome, which is an autosomal dominant condition that generally occurs *de novo*. Dravet syndrome is a severe early-onset epileptic encephalopathy, characterized by onset around six months of age of afebrile generalized tonic-clonic seizures with developmental regression in a previously well infant. Additional features include seizure types including prolonged febrile, hemiclonic, myoclonic, focal, and atypical absence seizures, abnormal interictal EEG, and progressive gait deterioration (Dravet, 2005, Ragona, 2011; Brunklaus, 2012; Rodda, 2012). Patients with Dravet syndrome may be at an increased risk of sudden unexpected death in epilepsy (SUDEP) compared to patients with other types of epilepsy (Skluzacek, 2011). GEFS+ is a mild epilepsy syndrome without intellectual disability characterized by febrile seizures that continue beyond the age when they are expected to resolve or the occurrence of both febrile and afebrile generalized tonic-clonic seizures before six years of age. Other commonly observed seizure types within GEFS+ families include myoclonic-atonic epilepsy, typical absence seizures, myoclonic seizures, and focal seizures (Scheffer, 1997; Escayg, 2000; Marini, 2007). Familial febrile seizures are characterized by simple febrile seizures that occur between 5 months and 4 years and resolve by 6 years of age (Mantegazza, 2005). Diagnostic criteria for familial hemiplegic migraine include migraine with aura, which involves recurring neurological symptoms localizable to the cerebral cortex or brain stem followed by headache, nausea, and/or photophobia. Aura symptoms may include reversible hemiparesis and visual, sensory, or speech disturbance (Thomsen, 2002; Jen, 2015). Gain of function has been reported as the mechanism of disease for familial hemiplegic migraines (Dhifallah, 2018).

Brunklaus A, *et al.* (2012) *Brain* **135**(8):2329-2336.
Dravet C, *et al.* (2005) *Adv Neurol* **95**:71-102.
Jen JC. (1993) GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2020. 2001 Jul 17 [updated 2015 May 14].
Scheffer IE & Berkovic SF (1997) *Brain* **120**(3):479-490.

Order Summary: The following products were included in the test order for this individual. Please Note: tests on hold and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) are excluded. For additional information, please contact Ambry Genetics.

- EpilepsyNext® (Product Code 6864)

Electronically Signed By Sample Director, on 8/25/2020 at 0:00:00 PM.

All content hereafter is supplemental information to the preceding report.

Report References

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- Dhifallah S, *et al.* (2018) *Front Mol Neurosci* **11**(0):232. **PMID:**30038559
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- Fujiwara T, *et al.* (2003) *Brain* **126**(0):531-46. **PMID:**12566275
- Ishii A, *et al.* (2017) *Epilepsia* **58**(2):282-290. **PMID:**28012175
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- Vahedi K, *et al.* (2009) *Neurology* **72**(13):1178-83. **PMID:**19332696

Genes Analyzed

ALDH7A1, AMT, ANKRD11, ARHGEF9, ARX, ASNS, ATP13A2, ATP1A2, ATP1A3, BRAT1, CACNA1A, CACNA1E, CASK, CDKL5, CHD2, CHRNA2, CHRNA4, CHRNB2, CLN3, CLN5, CLN6, CLN8, CNTNAP2, COL4A1, CSTB, CTSD, CTSF, DCX, DDC, DEPDC5, DNAJC5, DNM1, DYNC1H1, DYRK1A, EEF1A2, EHMT1, EPM2A, FLNA, FOLR1, FOXG1, FOXP1, GABRA1, GABRB3, GABRG2, GAMT, GATM, GLDC, GNAO1, GOSR2, GRIN1, GRIN2A, GRIN2B, GRN, H3F3A, HCN1, HNRNPU, IQSEC2, KCNA2, KCNB1, KCNC1, KCNH1, KCNJ10, KCNQ2, KCNQ3, KCNT1, KCTD7, KIAA2022, LGI1, MBD5, MECP2, MEF2C, MFSD8, MOCS1, MOCS2, NGLY1, NHLRC1, PACS1, PCDH19, PHGDH, PIGA, PLCB1, PNKP, PNPO, POLG, PPT1, PRICKLE1, PRRT2, PURA, RHOBTB2, SATB2, SCARB2, SCN1A, SCN1B, SCN2A, SCN3A, SCN8A, SIK1, SLC13A5, SLC19A3, SLC25A22, SLC2A1, SLC35A2, SLC6A1, SLC6A8, SLC9A6, SMC1A, SNAP25, SPTAN1, ST3GAL5, STX1B, STXBP1, SYNGAP1, SZT2, TBC1D24, TBL1XR1, TCF4, TPP1, TRIO, TSC1, TSC2, TUBA1A, UBE3A, WDR45 and ZEB2.

Metrics and Coverage

Complete coverage data for this proband is available for download through AmbryPort or can be e-mailed by request.

The following genes (coverage)* did not achieve 100% coverage at 10X for all nucleotides in the coding regions:

ANKRD11 (99.96%), ***ARX*** (89.64%), ***CACNA1A*** (99.93%), ***CDKL5*** (97.19%), ***FOXG1*** (93.13%), ***IQSEC2*** (97.09%), ***PURA*** (96.8%), ***SLC35A2*** (97.65%), ***SMC1A*** (99.19%), ***SYNGAP1*** (98.96%), ***TRIO*** (99.7%)

*percentage of the coding region covered at ≥10X

Assay Information

General Information: Recent literature regarding the genetics of the epilepsies has highlighted that one gene can be associated with multiple epilepsy syndromes; and one epilepsy syndrome can be associated with multiple genes. These findings strongly support the use of a broad multi-gene panel for genetic testing in the epilepsies, especially in individuals with an unclear history or atypical presentation.
EpilepsyNext® is an epilepsy panel that includes the most common genes known to cause seizures.

Methodology: Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's whole blood. Samples are prepared using the IDT xGen Exome Research Panel v1.0 (Integrated DNA Technologies). Each DNA sample is sheared, adaptor ligated, PCR-amplified and incubated with the exome baits. Captured DNA is eluted, and PCR amplified. Final quantified libraries are seeded onto an Illumina flow cell and sequenced using paired-end, 150 cycle chemistry on the Illumina HiSeq or NextSeq. Initial data processing, base calling, alignments and variant calls are generated by various bioinformatics tools. Data is annotated with the Ambry Variant Analyzer tool (AVA), including, but not limited to, the following information: nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency, and predicted functional impact. The following sites are used to search for previously described gene mutations and polymorphisms: The Human Gene Mutation Database (HGMD), the Online Mendelian Inheritance in Man (OMIM), the genome aggregation database (gnomAD), HapMap data, and online search engines (e.g., PubMed). Variants are filtered further based on likelihood of pathogenicity (Farwell K, et al., Genet Med., 2014), as well as by manual preliminary screening performed by licensed genetic counselors using criteria obtained from Ambry's General Variant Classification Scheme (https://www.ambrygen.com/science/variant-classification) to further filter alterations that are unlikely to be classified as disease-causing. The exome is targeted and sequenced, but analysis is limited to only the set of genes listed in this report. All reportable findings undergo manual review by molecular geneticists using integrated genomics software (IGV) and undergo confirmation either by automated fluorescence dideoxy (aka "Sanger") sequencing or via coverage and alternate read ratios above established confidence thresholds (heterozygous calls with 40-65% variant allele frequency and >40x coverage, hemizygous and homozygous calls with 100% variant allele frequency and >40x coverage). Co-segregation studies are performed if family members are available. Co-segregation results may be confounded by many factors which cannot be completely ruled out including reduced penetrance, age-of-onset, and/or variable expressivity. In most cases, phase cannot be determined.
New gene lists are regularly added due to proactive review of current literature using Ambry's peer-reviewed clinical validity scheme to ensure inclusion of the most up-to-date disease-associated genes (Smith ED, Radtke K, Rossi M, et al. 2017 Human mutation 38(5):600-608). As part of the Patient for Life program, Ambry will continually review past patient's data for potential mutations in newly added genes and proactively issue reclassification reports, as applicable.

Analytical range: Approximately 75% of the bases are expected to have quality scores of Q30 or higher, which translates to an expected base-calling error rate of 1:1000, or an expected base-calling accuracy of 99.9%. Additionally, 90% and 95% of the exome will be covered at ≥20x and ≥10x, respectively. Coverage is sufficient to detect >98% and up to 99.7% of disease-causing mutations (LaDuca H, Farwell KD, Vuong H, et al. 2017. PLoS ONE 12(2):e0170843). Coding exons plus at least 6 bases into the 5' and 3' ends of all the introns are analyzed and reported. Gross deletion/duplication analysis is assessed for all genes within the targeted exome using a custom pipeline based on coverage (>4 exons in size) and/or breakpoint analysis from NGS data and confirmed by targeted chromosomal microarray, SNP array or MLPA when applicable. CNVs detected by NGS pipeline for which no orthogonal method of confirmation is available will not be included.

Result Reports: In result reports, alterations in the following classifications are always reported, and are based on the following definitions and clinical recommendations:

- **Pathogenic Mutation:** Alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing for at-risk, affected, or possible carrier relatives may be warranted.
- **Variant, Likely Pathogenic (VLP):** alterations with strong evidence in favor of pathogenicity. Targeted testing for at-risk, affected, or possible carrier relatives may be warranted.
- **Variant, Unknown Significance (VUS): only if requested**, alterations with limited and/or conflicting evidence regarding pathogenicity will be reported.

Alterations of unlikely clinical significance (those with strong/very strong evidence to argue against pathogenicity) are not included on results reports. These include findings classified as "likely benign" and "benign" alterations.
A clinical report will only be generated for the proband, even when familial samples are received. Default reporting does not include variants of unknown significance (VUS), however clinicians may opt-in. If clinicians do not opt-in to receiving VUSs, family member samples will not be used for co-segregation analysis for alterations without the potential to be upgraded to VLP/Pathogenic.

Test Limitations and Disclaimer: This test was developed, and its performance characteristics were determined, by Ambry Genetics. It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be referred to a genetic counselor, medical geneticist, or physician skilled in evaluating the relevant medical literature. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. The following types of mutations are detectable: nucleotide substitutions, small deletions, small insertions, small indels, and copy number variants (CNV). The overall coverage of each genes varies and each individual may have slightly different coverage yield. Accurate exon-level CNV detection by exome sequencing is dependent on several factors such as inherent sequence properties of the targeted regions, including shared homology and exon size, depth-of-coverage, efficiency of capture, and degree of read depth variation in the selected background samples. Therefore, the specificity and sensitivity of CNV detection by exome sequencing maybe reduced. This assay is not is not intended to systematically detect and analyze, gross rearrangements, deep intronic variations, long repeat sequences, portions of genes with highly homologous pseudogenes, repeat expansions, mutations involved in tri-allelic inheritance, mitochondrial genome mutations, epigenetic effects, oligogenic inheritance, or X-linked recessive mutations in females who manifest disease due to skewed X-inactivation and other unknown abnormalities. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutation in the undetectable region. Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from rare genetic variants that may interfere with analysis, or from other sources. The clinical sensitivity of the test may vary widely according to the specific clinical and family history. Disorders of neurodevelopment are a complex spectrum of clinical disorders. Mutations in other genes or the regions not analyzed by this panel can also give rise to similar clinical conditions.

Resources: The following references are used in variant analysis and classification when applicable for observed genetic alterations.

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