Genetic Testing for Epilepsy: STAT Epilepsy Panel Sequence Analysis and Exon-Level Deletion/Duplication Testing of 26 Genes

Panel Gene List: ALDH7A1, ARX, BRAT1, CDKL5, FOLR1, GLDC, KCNQ2, KCNQ3, KCNT1, MECP2, MEF2C, PCDH19, PNPO, POLG, SCN1A, SCN1B, SCN2A, SCN8A, SLC19A3, SLC2A1, SLC6A8*, SPTAN1, STXBP1, TPP1, TSC1, TSC2

* This panel does not include deletion/duplication testing of the SLC6A8 gene

Clinical Features:

Epilepsy is defined by the occurrence of at least two unprovoked seizures occurring more than 24 hours apart. It is a common neurological disorder that affects at least 0.8% of the population. The International League against Epilepsy (ILAE) classifies seizures into two main categories.[1] **Generalized epileptic seizures** originate in and rapidly engage both cerebral hemispheres. Tonic-clonic, absence, myoclonic, clonic, tonic, and atonic seizures are all types of generalized seizures. **Focal seizures** originate from neuronal networks within a single hemisphere. Traditionally, focal seizures have been classified as “simple partial seizures,” which do not result in an alteration of consciousness, and “complex partial seizures,” which cause a change in behavior or consciousness. Some types of seizures, such as infantile spasms, do not fit into either category and remain unclassified. Seizures can be self-limiting or controlled by standard therapeutic treatments in some cases; however, individuals with epileptic encephalopathy have severe seizures that are refractory to treatment, leading to cognitive and behavioral impairment secondary to the epileptic activity. Epilepsy may be an isolated neurological symptom, or it may occur in association with other neurological symptoms or medical problems.[2] Some individuals with epilepsy are diagnosed with an electroclinical syndrome such as West syndrome or Ohtahara syndrome based on the presence of characteristic EEG findings and the clinical and family history.[1]

The STAT Epilepsy Panel at GeneDx is designed to provide a faster turn-around-time than other epilepsy panels and is ideal for patients with new onset or exacerbation of seizures where results of genetic testing may have implications for treatment and management. The panel includes genes with a high diagnostic yield such as *SCN1A, SCN2A, SCN8A, KCNQ2, CDKL5, STXBP1,* and *SLC2A1*, as well as other genes includes associated with generalized, focal and unclassified seizures.[37] Many of the genes on the panel also have implications for treatment and management. See Table 1 for a summary of genes with well-established treatment implications. Additionally, clinical trials evaluating new potential treatments may be available for individuals with pathogenic variants in some genes on this panel. Information on clinical trials can be found at www.clinicaltrials.gov.

Table 1: Impact of Genetic Test Results on Therapeutic Decision-Making[2,38]

Gene	Disorder	Treatment Implications
ALDH7A1	Pyridoxine-dependent epilepsy and folinic acid-responsive seizures	Seizures respond to treatment with supplemental vitamin B6 and/or folinic acid
FOLR1	Cerebral folate deficiency	Seizures respond to treatment with supplemental folinic acid
PNPO	Pyridoxyl 5'-phosphate dependent epilepsy	Seizures respond to treatment with supplemental pyridoxal 5-phosphate (PLP)
POLG	Alpers-Huttenlocher and other POLG-related disorders	Avoid valproic acid, which can induce or accelerate liver disease
SCN1A	Dravet syndrome and other SCN1A-related disorders	Consider treatment with valproate, clobazam, stiripentol, levetiracetam, topiramate. Avoid phenytoin, carbamazepine, and lamotrigine.
SLC2A1	Glucose transporter type 1 deficiency syndrome (Glut1-DS)	Seizures typically respond to ketogenic diet
SLC19A3	Thiamine metabolism dysfunction disorders	Responds to treatment with biotin and thiamine supplementation
TSC1 TSC2	Tuberous sclerosis complex	Infantile spasms typically respond to treatment with vigabatrin

Inheritance Pattern/Genetics:

Epilepsy can be caused by genetic disorders, metabolic diseases, trauma, infection, and structural brain abnormalities, although the cause is not known in many cases. A genetic etiology underlies epilepsy in approximately 40% of individuals.[3] Genes have been identified that cause both generalized seizures and focal seizures, as well as unclassified epilepsy types such as infantile spasms. The genetic etiology of idiopathic generalized epilepsy (IGE) is frequently complex because it is due to a combination of multiple genetic factors that each confer a small risk for epilepsy and may be modified by environmental influences.[3] Currently, approximately 2% of patients with IGE harbor an identifiable variant in a single gene associated with Mendelian inheritance of epilepsy.[4] However, the percentage of patients with Mendelian epilepsy is higher for specific epilepsy types such as infantile spasms, Dravet syndrome, benign familial neonatal and neonatal-infantile seizures (BFNS and BFNIS), generalized epilepsy with febrile seizures plus (GEFS+), and others.[3,5,6,7] The inheritance pattern can be autosomal dominant, autosomal recessive, or X-linked. Pathogenic variants in a single gene may be associated with different types of seizures (clinical heterogeneity), and conversely, pathogenic variants in different genes can cause the same epilepsy phenotype (genetic heterogeneity).

The STAT Epilepsy Panel at GeneDx includes genes that encode subunits of ion channels involved in stabilizing or propagating neuronal activity, including components of the voltage-gated sodium and potassium channels.[5,7,8,9] The panel also includes non-ion channel genes

associated with neurotransmitter and other neurometabolic disorders, as well as genes causing syndromic forms of epilepsy, many of which are involved in transcriptional activation or repression.[5,6,8,10,11,12,38] The complete list of genes and associated disorders is included in Table 2 below.

Test Methods:
Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions of the genes on this panel are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

The technical sensitivity of sequencing is estimated to be > 99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size. For the SLC6A8 gene(s), sequencing but not deletion/duplication analysis, is performed.

If the STAT Epilepsy Panel is negative, sequencing and deletion/duplication analysis of the remaining genes on the Comprehensive Epilepsy Panel is available as a separate test.

Test Sensitivity:
The clinical sensitivity of sequencing and deletion/duplication analysis of the genes included in the STAT Epilepsy Panel depends in part on the patient's clinical phenotype. In a prior study, 31% of individuals with infantile spasms who were tested using an epilepsy gene panel were found to harbor definitive pathogenic variant(s) to explain the phenotype.[35] Overall, 17-20% of epileptic encephalopathies have an identifiable genetic etiology.[36] Specific information about the diagnostic yield for each gene in selected populations is summarized in the table below.

Table 2: Diagnostic Yield of Genes on STAT Epilepsy Panel

Epilepsy Type	Gene	Protein	Inh	Diagnostic Yield in Selected Population(s)
Benign familial neonatal seizures (BFNS)	KCNQ2	Potassium voltage-gated channel subfamily KQT member 2	AD	>50% BFNS[1]
	KCNQ3	Potassium voltage-gated channel subfamily KQT member 3	AD	~7% BFNS[1]
Benign familial neonatal-infantile seizures (BFNIS)	SCN2A	Sodium channel protein type 2 subunit alpha	AD	Unknown[1]
Early-onset epileptic encephalopathy and/or infantile spasms (includes West and Ohtahara syndromes)	CDKL5	Cyclin-dependent kinase-like 5	XL	10-17% infantile spasms[1]
	ARX	Aristaless related homeobox	XL	5% males with infantile spasms[1]
	TSC1	Hamartin	AD	2-4% infantile spasms[27,28,29]
	TSC2	Tuberin	AD	10-16% infantile spasms[27,28,29]
	SCN1A	Sodium channel protein type 1 alpha	AD	70-80% Dravet syndrome[1] 20-24% early-onset cryptic epilepsy[4,5]
	PCDH19	Protocadherin-19	XL	2-14% females with infantile/childhood epilepsy[6,7,8,9,10]
	KCNQ2	Potassium voltage-gated channel subfamily KQT member 2	AD	10% neonatal epileptic encephalopathy[11]
	STXBP1	Syntaxin binding protein 1	AD	35% Ohtahara syndrome[1]; Unknown in Dravet syndrome[12]
	SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1	AD	91% GLUT1 deficiency[2]; ~10% early-onset absence epilepsy[1]
	ALDH7A1	Alpha-aminoadipic semialdehyde dehydrogenase (antiquitin)	AR	>90% pyridoxine-responsive epilepsy[13]
	POLG	DNA polymerase subunit gamma-1	AR	63-87% Alpers syndrome[14,15,16]; 4-5% infantile/childhood epileptic encephalopathy[15]
	SCN2A	Sodium channel protein type 2 alpha	AD	1-2% early-onset epileptic encephalopathy[17,18]
	SCN8A	Sodium channel protein type 8 subunit alpha	AD	Unknown[19]
	SPTAN1	Alpha-II spectrin	AD	Rare[20]
	KCNT1	Potassium channel, sodium activated subfamily T, member 1	AD	35% MMPSI[30-32]; Rare in other epileptic encephalopathies[30]
	BRAT1	BRCA1 associated ATM activator 1	AR	Rare[39]
	PNPO	Pyridoxine-5'-phosphate oxidase	AR	Rare[21]
Generalized epilepsy with febrile seizures plus (GEFS+)	SCN1A	Sodium channel protein type 1 alpha	AD	5-10% GEFS+[1]
	SCN1B	Sodium channel subunit beta-1	AD	<5% GEFS+[1]
	SCN2A	Sodium channel protein type 2 alpha	AD	Rare[3]

Rett/atypical Rett syndromes	MECP2	Methyl CpG binding protein 2	XL	88% females with Rett syndrome[22]
	CDKL5	Cyclin-dependent kinase-like 5	XL	2-8% females with atypical Rett syndrome[23,24]
	MEF2C	Myocyte-specific enhancer factor 2C	AD	Rare in Rett-like syndromes[25]
Cerebral folate deficiency	FOLR1	Folate receptor alpha	AR	Rare[26]
Creatine transporter deficiency	SLC6A8*	Solute carrier family 6 (neurotransmitter transporter), member 8	XL	2% males with epilepsy and intellectual disability[33]; 65% males with biochemical creatine deficiency[34]
Glycine encephalopathy (also called nonketotic hyperglycinemia)	GLDC	Glycine decarboxylase	AR	70-75% glycine encephalopathy[40]
Thiamine metabolism dysfunction disorders	SLC19A3	Solute carrier family 19 member 3	AR	Rare[38]
Neuronal ceroid lipofuscinosis (NCL)	TPP1 CLN2	Tripeptidyl-peptidase 1	AR	95% TPP1 deficiency[41]

* Does not include deletion/duplication testing of SLC6A8

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