Clinical results from a pediatric neurological region of interest using an orthogonal NGS approach for identifying variants

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CLARITAS GENOMICS

Abstract

Statement of Purpose: There are many pediatric neurologic disorders that present with unique or overlapping phenotypes that often make identifying the underlying genetic variant a multi-year journey when using traditional molecular approaches to identify them. Through the use of next-generation sequencing (NGS) technologies, it is possible to sequence all the protein coding exons in the affected patient's genome simultaneously to quickly identify potential causative variants. In a clinical setting, it remains necessary to verify putative NGS results with additional molecular methods. In the case of whole exome sequencing, the number of variants needing confirmation may be in the hundreds.

Methods: At Claritas Genomics, we have developed an approach to clinical testing which limits the number of potential variants needing Sanger confirmation. This approach both focuses on a neurological region of interest (ROI) encompassing 614 genes in version 1 (1067 genes in version 2) that have been implicated in causing seven pediatric disorder categories as well as employing two NGS technologies to orthogonally detect and confirm variants in the neurological genes. Any variants that are not confirmed using this approach can be resolved by Sanger sequencing.

Results: We have compared results obtained using our assay with results from another provider's whole exome assay for 9 independent samples chosen for their pediatric neurological indications. These analyses were carried out in a blinded fashion. Of the nine samples, four samples produced clinically inconclusive findings in both assays. Three samples produced the same results in both assays. One sample produced a result that was interpreted as clinically relevant in the other provider's analysis but our analysis indicated this gene was not phenotypically relevant to the patient's disorder. The final sample produced a result that was clinically relevant based on our analysis but it was not identified in the other provider's whole exome. The use of orthogonal sequencing platforms provides immediate confirmation of most variants and also yields better sensitivity than either platform alone can generate, providing better results and more timely information for patients seeking answers to their diagnostic questions.

Neurological ROI is sequenced at high depth in both platforms

	70 genes from an Alternate provider's *Comprehensive Epilepsy Panel*	All 276 Epilepsy/ Seizure genes on Pediatric *Neurological Exome*	All 614 genes on Pediatric *Neurological Exome*
Genes	70	276	614
Exonic regions	850	4,437	10,651
Target Span	175 Kb	802 Kb	1.98 Mb
Mean target coverage, Proton	150x	145x	144x
Mean target coverage, Illumina	100x	87x	89x
Percent of targeted bases covered at ≥20x by either Proton or Illumina :	98.70%	98.60%	98.40%
Sensitivity (compared to NA12878 NIST reference)	100% (36/36)	97.5% (294/302)	98.3% (784/798)
Percent of Variants Orthogonally Confirmed:	80%	79%	82%
PPV for Orthogonally Confirmed Variants:	100% (31/31)	100% (246/246)	100% (675/675)

Orthogonal Sequencing Improves Data Quality

Orthogonal sequencing using independent target enrichment and sequencing methods leads to 100% PPV with NA12878 for variants found on both platforms and increases exome assay sensitivity to >98%. Variants found on only one platform are confirmed prior to reporting.

97% of targeted regions across whole exome are covered at >20x via NextSeq or Proton

Platform	Sensitivity	PPV
Illumina NextSeq	97.6%	99.6%
Ion Proton	94.7%	99.6%
Combined	99.0%	99.5%

Pediatric Neurological Exome Variants Confirmed Orthogonally with 100% PPV

Pediatric Neurological Exome		Total Variants	FP	TP	% Total TP	Cumulative Sensitivity	Cumulative Specificity (FP/Mb)	PPV
Orthogonally Confirmed, Both Platforms High Quality	All	821	0	821	87.5	86.1	0.0	100.0
	InDels	13	0	13	1.4	35.1	0.0	100.0
	SNVs	808	0	808	86.1	88.2	0.0	100.0
Reliable, Same call on both platforms, NoPass on Illumina	All	24	0	24	2.6	88.6	0.0	100.0
	InDels	0	0	0	0.0	35.1	0.0	NA
	SNVs	24	0	24	2.6	90.7	0.0	100.0
Likely True Positive, Call on one platform only or zygosity differences	All	106	13	93	9.9	98.6	8.9	87.7
	InDels	20	3	17	1.8	82.0	2.1	85.0
	SNVs	86	10	76	8.1	99.3	6.8	88.4
All Variant Categories	All	951	13	938	100.0	98.6	8.9	98.6
	InDels	33	3	30	3.2	82.0	2.1	90.9
	SNVs	918	10	908	96.8	99.3	6.8	98.9

Phenotypes covered in the *Pediatric Neurological Exome*

Version 1

Disorder Category	# of Genes
Neuromuscular disorder	297
Movement disorder	39
Epilepsy/Seizure	276
Brain Malformation	67
Heriditary Peripheral Neuropathy	84
Leukodystrophy/Encephalomyopathy	61

Version 2

Disorder Category	# of Genes
Neuromuscular disorder	549
Movement disorder	503
Epilepsy/Seizure	442
Brain Malformation	403
Heriditary Peripheral Neuropathy	267
Leukodystrophy/Encephalomyopathy	267

Comparison of *Pediatric Neurological Exome (V1)* to Whole Exome Sequencing

	Pathogenic finding, same variant?		additional	Claritas Rapid Report	Claritas Complete
Case	Alternate	Claritas		Compared to additional Alternate reported variants	Additional Claritas variants, related to phenotype
1	Yes GBE1	Yes GBE1	1 VUS in GUS, 1 Path, non-neuro	Additional Alternate variants not neuro-related, not on Claritas test	5 VUS (not same), 1 additional neuro Path	
2	Yes KAT6B	Yes KAT6B (in Complete Report)	1 likely Path COL8A2	COL8A2 (corneal dysfunction dystrophy), not on Claritas test	6 VUS	Path variant KAT6B
3	Yes CHD2	Yes CHD2		Also found a second neuro Path: BTD	4 VUS	
5	Yes KMT2A	no		KMT2A (short stature) Not clear implication in neuro, not on current Claritas test	6 VUS	1 VUS
4	No	Yes GPR98	1 VUS, 7 data from HGMD (one was GPR98)	1 Path (GPR98) and 1 likely path not found on Alternate, VUS was not , 2 HGMD were benign, others not neuro, not on Claritas test	5 VUS (not same as Alternate)	2 VUS (not same as Alternate)
6	no	no	2 VUS, 1 unrelated Path	2 VUS (Saw both Alternate VUS, reported one). Unrelated Path is melanoma predisposition, not on Claritas test	1 VUS	
7	no	no	1 likely Path CHRNA1	Same CHRNA1 variant, but classified as uncertain.	9 VUS	
8	no	no	3 VUS	#1: same VUS, #2: not neuro, not on Claritas test, #3: low coverage both platforms	5 VUS, 1 neuro Path, but not pheno	2 VUS
9	no	no	1 VUS	VUS not neuro-related, not on Claritas test	6 VUS	

Rapid Report

Results Summary

Positive
Clinically Relevant Results

Phenotypic Summary

At the time of sample submission, your patient's clinical features did not seem to fit a recognizable syndrome and therefore, based on the complex neurological phenotypes, you ordered the *ClariFocus Exome* for Pediatric Neurology. You described your patient as having the following clinical features:

- Developmental delay
- Generalized hypotonia
- Agenesis of the corpus callosum
- Asymmetry of the face and lower limbs
- Bilateral microcornea
- Tracheobronchomalacia
- Hepatosplenomegaly

You feel the clinical features observed in your patient may have a common underlying molecular diagnosis; however, in your opinion the primary neurological features in this individual are muscular hypotonia, developmental delay, and agenesis of the corpus callosum.

Synopsis

The *ClariFocus Exome* for Pediatric Neurology: Rapid Report contains orthogonally-confirmed variants (identified on two independent Next Generation sequencing platforms) in genes related to the patient's indicated phenotype. In addition, this report also contains likely pathogenic and pathogenic orthogonally-confirmed variants in genes not obviously related to the patient's indicated phenotype. Based on the phenotypic information provided, the Human Phenotype Ontology (HPO) terms neurodevelopmental delay (HP:0012758), muscular hypotonia (HP: 0001252), and abnormality of forebrain morphology, which captures genes associated with agenesis of the corpus callosum (HP:0100547), were used to filter variants for the Rapid Report (see Genes with Known Relevance to Indicated Phenotype(s) for details). Six variants in six genes related to the patient's indicated phenotype were identified from this analysis (see Results Related to Indicated Phenotype for details). This includes a homozygous pathogenic variant in *GBE1* which has been previously reported in individuals with adult polyglucosan body disease. Despite the adult onset of this condition this finding is consistent with a molecular diagnosis of a *GBE1*-related disorder.

Results

Related to Indicated Phenotype

Confirmed pathogenic, likely pathogenic, and variants of uncertain significance occurring in genes related to the indicated phenotype(s) are listed below:

#	Classification	Gene	Nucleotide change	Amino acid change	Variant type	Zygosity	Transcript
1	Pathogenic	*GBE1*	c.1544G>A	p.Arg515His	Missense	Homozygous	NM_000158.3
2	Uncertain	*CEP290*	c.3773A>G	p.Asn1258Ser	Missense	Heterozygous	NM_025114.3
3	Uncertain	*L1CAM*	c.2930C>T	p.Thr977Ile	Missense	Hemizygous	NM_024003.3
4	Uncertain	*PQBP1*	c.292+6T>G	n/a	Intronic	Hemizygous	NM_005710.2
5	Uncertain	*SLC25A22*	c.267C>G	p.Phe89Leu	Missense	Heterozygous	NM_024698.5
6	Uncertain	*SPG11*	c.1939A>G	p.Ile647Val	Missense	Heterozygous	NM_025137.3

GBE1

1 The c.1544G>A (p.Arg515His) variant in *GBE1* is previously reported in association with glycogen branching enzyme (GBE) deficiency causing adult polyglucosan body disease (APBD). This variant has been reported in a homozygous or compound heterozygous state in multiple individuals with adult polyglucosan body disease. The clinical presentation of these individuals was notably different from those with classic glycogen storage disease type IV, which is also associated with pathogenic variants in *GBE1*. Affected individuals with this variant reportedly had decreased GBE activity and adult onset of episodic neurological symptoms, including gait difficulties, peripheral neuropathy, and neurogenic bladder as well as mild cognitive difficulties[1,2]. Childhood onset liver manifestations were also reported in a subset of individuals with the c.1544G>A (p.Arg515His) variant; these individuals may represent an intermediate form of GBE deficiency, given their atypical collection of manifestations[3]. A different amino acid change at p.Arg515, a c.1543C>T (p.Arg515Cys), has been reported in an individual with features of classic glycogen storage disease type IV[4]. Therefore, based on the previous reports in the literature, this variant is considered to be pathogenic.

Additional Claritas Genomics Posters:

Program Number 1981
Title: Improved Sensitivity and Rapid Confirmation of Variants via Orthogonal Sequencing of Exomes
Presenter: John Thompson, Ph.D. Chief Technology Officer, Claritas Genomics
Location: Convention Center, Exhibit Hall, Level 1
Time/Date: Wednesday, 5 PM – 6 PM

Program Number 1933
Title: Strategies for calculating variant confidence by combining sequencing results
Presenter: Niru Chennagiri
Location: Convention Center, Exhibit Hall, Level 1
Time/Date: Wednesday, 5 PM – 6 PM

Program Number 2070
Title: Next-Generation Sequencing test within a neurologic region of interest leads to diagnosis of RYR1-related disorder for 36-year-old female after three decades
Presenters: Pam Gerrol, Claritas Genomics
Location: Convention Center, Exhibit Hall, Level 1
Time/Date: Friday, 11:45 AM – 12:45 PM

Program Number 1619
Title: Improving Specificity in Ion Proton Data
Presenter: Daniel Lieber, Ph.D. TITLE, Claritas Genomics
Location: Convention Center, Exhibit Hall, Level 1
Time/Date: Thursday, 11 AM – 12 PM

Program Number 2085
Title: UPD1 in a Newborn with Multiple Congenital Anomalies
Presenters: Ann Seman , Claritas Genomics, L. Rodan and A. C. Woerner, Boston Childrens Hospital
Location: Convention Center, Exhibit Hall, Level 1
Time/Date: Friday, 10:45 AM – 11:45 AM

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