

Patient Name: [REDACTED]
Date of Birth: [REDACTED]
Gender:
Hospital/MR #:
Accession #:
Sample Type: EXT DNA
Test Code: 1500
Indication: Symptomatic

Lab Number: [REDACTED]
Family #: [REDACTED]
Date Collected:
Date Received: November 1, 2013
Date Ordered:
Date Reported: February 16, 2015

Yaping Yang, PhD.
Medical Genetics Labs/DNA
Tel No. : 713-798-6555
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CC: MGL Internal Provider, Fax # : 713-798-4187

Whole Exome Sequencing Version 3: Focused Report



HETEROZYGOUS DE NOVO MUTATION IN ARID1B GENE DETECTED, RELATED TO PATIENT'S CLINICAL PHENOTYPE

According to clinical information submitted by the referring center, this individual has delayed motor milestones, delayed speech, autism, intellectual disability, seizures and dysmorphic features. Prior work-up was unrevealing. We were requested to perform whole exome sequencing on DNA extracted from a whole blood sample from this individual. We also received blood samples from the mother (sample #XXXXXX) and father (sample #YYYYYY) of this individual,

1. Pathogenic variants in disease genes related to clinical phenotype (Table 1)

A novel heterozygous c.2029dupA (p.S677fs) mutation in the ARID1B gene was detected in this individual. This mutation is categorized as deleterious according to guidelines (ACMG Recommendations for Standards for Interpretation and Reporting of Sequence Variations: Revision 2007. Genet Med 2008;10:21844). Defects in ARID1B are the cause of mental retardation autosomal dominant type 12 (MRD12) [MIM:614562]. MRD12 patients present with moderate to severe psychomotor retardation, and most show evidence of muscular hypotonia. In many patients, expressive speech is more severely affected than receptive function. Additional common findings include short stature, abnormal head shape and low-set, posteriorly rotated, and abnormally shaped ears, downslanting palpebral fissures, a bulbous nasal tip, a thin upper lip, minor teeth anomalies, and brachydactyly or single palmar creases. Autistic features are uncommon. This individual is heterozygous for this mutation, which was confirmed by Sanger sequencing of the targeted region of the ARID1B gene. Sanger sequencing analysis of this region in the parental samples submitted for this individual did not detect the c.2029dupA (p.S677fs) change, suggesting that this change arose de novo in this patient. However, the possibility of mosaicism in either parent cannot be excluded. Our interpretation is premised upon the assumption that we have tested the biological parental samples of this patient.

The finding of a deleterious mutation in the ARID1B gene of this individual may be consistent with the described phenotype. It is recommended that correlation of these findings with the clinical phenotype be performed.

2a. Likely pathogenic variants in disease genes related to clinical phenotype (Table 2a)

None detected by WES

2b. Variants of unknown clinical significance in disease genes related to clinical phenotype (selected variants are discussed below, see Table 2b for the complete list)

a. A heterozygous c.1124C>T (p.S375L) variant of unknown clinical significance (VUS) in the KANSL1 gene was detected in this individual. Defects in KANSL1 are the cause of autosomal dominant Koolen-De-Vries syndrome [MIM: 610443], a syndrome characterized by moderate to severe intellectual disability, hypotonia, friendly demeanor, and highly distinctive facial features, including tall, broad forehead, long face, upslanting palpebral fissures, epicanthal folds, tubular nose with bulbous nasal tip, and large ears. More variable features include cardiac or genitourinary anomalies and seizures. This individual is heterozygous for this VUS, which was confirmed by Sanger sequencing. Sanger sequencing also showed that the mother is heterozygous for this change, the father is negative.

b. A heterozygous c.95A>C (p.Y32S) variant of unknown clinical significance (VUS) in the CDH15 gene was detected in this individual. Defects in CDH15 are the cause of mental retardation autosomal dominant type

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3 (MRD3) [MIM:612580]. Mental retardation is characterized by significantly sub-average general intellectual functioning associated with impairments in adaptive behavior and manifested during the developmental period. This individual is heterozygous for this VUS, which was confirmed by Sanger sequencing. Sanger sequencing also showed that the mother is heterozygous for this change, the father is negative.

It is recommended that correlation of these findings with the clinical phenotype be performed.

3. Medically actionable pathogenic variants in disease genes unrelated to clinical phenotype (Table 3):

A heterozygous c.8548_8551del(p.E2850fs) mutation in the BRCA2 gene was detected in this individual. This mutation has been previously reported in a study of hereditary breast and ovarian cancer [PMID: 21120943]. Defects in BRCA2 are a cause of susceptibility to breast cancer (BC) [MIM: 114480], pancreatic cancer type 2 (PNCA2) [MIM: 613347], Fanconi anemia complementation group D type 1 (FANCD1) [MIM: 605724] and Wilms tumor [MIM: 194070]. This individual is heterozygous for this mutation, which was confirmed by Sanger sequencing. Sanger sequencing showed that the father is heterozygous for this change, the mother is negative. Clinical correlation and genetic counseling for the patient and at-risk family members is recommended for the medically actionable mutation. BRCA2 familial studies can be performed at a laboratory licensed for BRCA2 testing. Genetic counseling for medically actionable mutations is recommended.

4. Carrier Status for Recessive Mendelian Disorders (Table 4):

A heterozygous deleterious mutation predicting carrier status was detected in this individual for an autosomal recessive disorder. Clinical correlation and genetic counseling for the mutation(s) conferring carrier status for autosomal recessive conditions is recommended.

5. Pharmacogenetic Results (Table 5)

See Table 5.

RECOMMENDATION SUMMARY:

It is recommended that correlation of these findings with the clinical phenotype be performed. Genetic counseling for medically actionable mutations is recommended. Clinical correlation and genetic counseling for the mutation(s) conferring carrier status for autosomal recessive conditions is recommended. Please note that the focused report is limited to genes predicted to be related to the clinical phenotype, medically actionable mutations, as well as carrier status for autosomal recessive conditions recommended for carrier screening by professional organizations such as ACMG or ACOG. An expanded report of the Whole Exome Sequencing Test is available. The Expanded Report will give additional information on mutations and variants in genes which cause disease unrelated to the indication for testing and predicted deleterious mutations in genes with no known current association with disease. It is available at no additional cost if requested within 6 months of the date of the Focused report. The turn-around time for the Expanded Report is 4 weeks. To order, please visit our website at www.bcmgeneticlabs.org and complete the Whole Exome Expanded Report Requisition (Test Code 1510) and FAX to 713-798-2787

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RESULT TABLES

Table 1: Pathogenic Variants in Disease Genes Related to Clinical Phenotype

Disease	Inheritance Pattern	Gene	Position	Isoform	Location	Nucleotide	Amino Acid	Zygoty	References/Comments	EVS AA/EA	SIFT / PolyPhen-2
Mental retardation, autosomal dominant 12 [MIM:614562]	AD	ARID1B	Chr6: 157256700	NM_020732.3	exon5	c.2029dupA	p.S677fs	Het	Novel mutation. Confirmed by Sanger sequencing. Mother and father are negative.	N/R N/R	N/A/ N/A

Table 2a. Likely Pathogenic Variants in Disease Genes Related to Clinical Phenotype

None detected by WES

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Table 2b. Variants of Unknown Clinical Significance in Disease Genes Related to Clinical Phenotype

Disease	Inheritance Pattern	Gene	Position	Isoform	Location	Nucleotide	Amino Acid	Zygoty	References/Comments	EVS AA/EA	SIFT / PolyPhen-2
Koolen-De Vries syndrome [MIM:610443]	AD	KANSL1	Chr17: 44248386	NM_001193466	exon2	c.1124C>T	p.S375L	Het	rs142696045. Confirmed by Sanger sequencing. Mother is heterozygous. Father is negative.	1/3737 2/7018	Tolerated/ Probably damaging
Mental retardation, autosomal dominant 3 [MIM:612580]	AD	CDH15	Chr16: 89245876	NM_004933	exon2	c.95A>C	p.Y32S	Het	Novel variant. Confirmed by Sanger sequencing. Mother is heterozygous. Father is negative.	N/R N/R	Tolerated/ Benign
Ceroid lipofuscinosis, neuronal, 8 [MIM:600143]; Ceroid lipofuscinosis, neuronal, 8, Northern epilepsy variant [MIM:610003]	AR	CLN8	Chr8: 1719270	NM_018941	exon2	c.50A>G	p.D17G	Het	rs148668081	3/3735 0/7020	Damaging/ Probably damaging
Hypomagnesemia 1, intestinal [MIM:602014]	AR	TRPM6	Chr9: 77418710	NM_017662	exon15	c.1731G>A	p.K577K	Het	Novel variant	N/R N/R	N/A/ N/A

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Hypomagnesemia 4, renal [MIM:611718]	AR	EGF	Chr4: 110901983	NM_001963	exon15	c.2223A>G	p.G741G	Het	rs141644661	0/3738 6/7014	N/A/ N/A
Stocco dos Santos X-linked mental retardation syndrome [MIM:300434]	X-linked	SHROOM4	ChrX: 50377532	NM_020717	exon4	c.1541G>T	p.R514I	Het	rs149678940. Confirmed by Sanger sequencing. Mother is heterozygous. Father is negative.	0/3216 1/5544	Damaging/ N/A

Table 3: Medically Actionable Pathogenic Variants in Disease Genes Unrelated to Clinical Phenotype

Disease	Inheritance Pattern	Gene	Position	Isoform	Location	Nucleotide	Amino Acid	Zygoty	References/Comments	EVS AA/EA	SIFT / PolyPhen-2
Breast-ovarian cancer, familial, 2 [MIM:612555]; Breast cancer, male, susceptibility to [MIM:114480]; Fanconi anemia, complementation group D1 [MIM:605724]; Pancreatic cancer [MIM:613347]; Prostate	AD	BRCA2	Chr13: 32945149	NM_000059	exon20	c.8548_8551del	p.E2850fs	Het	PMID 21120943; Confirmed by Sanger;	N/R N/R	N/A/ N/A

Table 4: Carrier Status for Recessive Mendelian Disorders

Disease	Inheritance Pattern	Gene	Position	Isoform	Location	Nucleotide	Amino Acid	Zygoty	References/Comments	EVS AA/EA	SIFT / PolyPhen-2
Gaucher disease, perinatal lethal [MIM:608013]; Gaucher, type I [MIM:230800]; Gaucher, type II [MIM:230900]; Gaucher, type III [MIM:231000]; Gaucher, type IIIC [MIM:231005]	AR	GBA	Chr1: 155205634	NM_001005741	exon10	c.1226A>G	p.N409S	Het	Confirmed by Sanger sequencing. Common mutation, first reported in PMID 3353383; rs76763715; legacy name p.N370S;	0/3738 22/6996	Damaging/ Benign

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Table 5: Pharmacogenetic Profile Variant Alleles

Drug	Allele	Zygoty	References/Comments
Anti-depressants, anti-epileptics, Plavix	CYP2C19*2	Het	Non-functional allele, PMID 8195181, www.pharmgkb.org/gene/PA124, CYP2C19*2; rs4244285
Anti-depressants, anti-epileptics, Plavix	CYP2C19*17	Het	Increased activity, PMID 16413245, www.pharmgkb.org/gene/PA124; rs12248560
Warfarin	VKORC1-1639G>A	Het	Decreased expression, PMID 21900891, www.warfarindosing.org, www.pharmgkb.org/gene/PA133787052; rs9923231

METHODOLOGY:

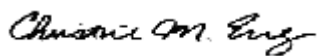
- Whole exome sequencing (WES)*: for the paired-end pre-capture library procedure, genome DNA is fragmented by sonicating genomic DNA and ligating to the Illumina multiplexing PE adapters. The adapter-ligated DNA is then PCR amplified using primers with sequencing barcodes (indexes). For target enrichment/exome capture procedure, the pre-capture library is enriched by hybridizing to biotin labeled VCRome 2.1 in-solution exome probes (reference 3) at 47°C for 64 - 72 hours. Additional probes for over 3600 Mendelian disease genes were also included in the capture in order to improve the exome coverage. For massively parallel sequencing, the post-capture library DNA is subjected to sequence analysis on Illumina HiSeq platform for 100 bp paired-end reads. The following quality control metrics of the sequencing data are generally achieved: >70% of reads aligned to target, >95% target base covered at >20X, >85% target base covered at >40X, mean coverage of target bases >100X. SNP concordance to genotype array: >99%. This test may not provide detection of certain genes or portions of certain genes due to local sequence characteristics or the presence of closely related pseudogenes. Gross deletions or duplications, changes from repetitive sequences may not be accurately identified by this methodology.
- As a quality control measure**, the individual's DNA is also analyzed by a SNP-array (Illumina HumanExome-12v1 array). The SNP data are compared with the WES data to ensure correct sample identification and to assess sequencing quality.
- Data analysis and interpretation by Mercury 1.0**: The output data from Illumina HiSeq are converted from bcl file to FastQ file by Illumina CASAVA 1.8 software, and mapped by BWA program. The variant calls are performed using Atlas-SNP and Atlas-indel developed in-house by BCM HGSC. The variant annotations are performed using in-house developed software: HGSC-SNP-anno and HGSC-indel-anno. Synonymous variants, intronic variants not affecting splicing site, and common benign variants are excluded from interpretation unless they were previously reported as pathogenic variants. The variants were interpreted according to ACMG guidelines (reference 5) and patient phenotypes. Variants related to patient phenotypes are usually confirmed by Sanger sequencing for patients and if available, parents. Sanger confirmation is noted in the "References/Comments" section of the tables if performed. It should be noted that the data interpretation are based on our current understanding of genes and variants at the time of reporting.
- The focused report contains results of genes related to the patient's clinical phenotype. This interpretation is based on the clinical information provided by the referring center. Results reported in the focused report should be carefully correlated to the individual's clinical findings by the referring physician. Results of carrier status for autosomal recessive conditions are limited to disorders which are recommended for carrier screening by professional societies such as ACMG or ACOG. Pharmacogenetic variants are limited to CYP2C9*2, CYP2C9*3, CYP2C9*5, CYP2C9*6, VKORC1-1639G>A, CYP2C19*2, CYP2C19*3, CYP2C19*4, CYP2C19*5, CYP2C19*8, CYP2C19*10, and CYP2C19*17.
- The WES report includes minor allele frequency (MAF) data for variants reported in the Exome Variant Server (EVS) database from NHLBI GO Exome Sequencing Project (ESP) (<http://evs.gs.washington.edu/EVS/>). For example, "1/3738 3/7017" in the MAF column labeled as "EVS AA/EA" means in the current EVS database which contains about 6500 individuals, for the variant listed, the minor allele was observed one time and the major allele was observed 3738 times in African Americans (AA), while the minor allele was observed three times and the major allele was observed 7017 times in Europeans (EA). N/R in the column denotes "Not Reported". Earlier samples utilizing data from an earlier version of the EVS database with about 5400 individuals have the MAF column labeled as "ESP5400 AA/EA".
- The WES report also includes in silico predictions for nonsynonymous (missense) changes by SIFT (reference 6) and PolyPhen-2 (reference 7). The predictions are listed in the "SIFT/PolyPhen-2" column with

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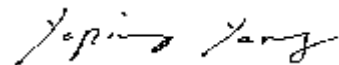
predictions from SIFT (Damaging, Tolerated or N/A) listed first followed by predictions from PolyPhen-2 (Probably damaging, Possibly damaging, Benign or N/A). N/A denotes predictions that cannot be obtained. The N/A situation applies to nonsynonymous changes for which the algorithm used failed to output a prediction or changes not classified as nonsynonymous, for example, nonsense or frameshift changes. It should be noted that in silico prediction results may vary depending on the algorithm and databases used at the time and should only be used as a guide but not solely relied upon for variant classifications.

References:

1. Illumina, Inc. (2011) Multiplexing Sample Preparation Guide (Part # 1005361 Rev. D). 2011.
2. Roche NimbleGen, Inc. (2010) NimbleGen SeqCap EZ Exome Library SR User's Guide (Version 2.2).
3. Bainbridge MN, Wang M, Wu Y, Newsham I, Muzny DM, Jefferies JL, Albert TJ, Burgess DL, Gibbs RA (2011). Targeted Enrichment beyond the Consensus Coding DNA Sequence Exome Reveals Exons with Higher Variant Densities. Genome Biology 12(7):R68. PMID:21787409.
4. Li H. and Durbin R. (2009) Fast and accurate short read alignment with Burrows-Wheeler Transform. Bioinformatics, 25:1754-60. PMID:19451168.
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7. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR (2010). PolyPhen-2. Nat Methods 7:248-249. PMID:20354512.



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