alamya health BREAKTHROUGH GENOMICS

Patient Information		Laboratory		Provider Information	
Patient No.:		Sample ID:	ABC123-1	Ordering Provider	Name
Patient Name:	First Last	Specimen:	Whole blood		Organization
Date of Birth:	YYYY-MM-YY	Sample Collected:	YYYY-MM-DD		Email/Phone/Fax
Sex:		Sample Received:	YYYY-MM-DD	Copied	Name (Email)
Race/Ethnicity:		Report Date:	YYYY-MM-DD		

LABORATORY REPORT
Proband Analysis

Indication for testing	Individual with developmental delay and dysmorphic facies with a history of gastroesophageal reflux, hemiplegia, seizure and stroke.
Test requested	Clinical Whole Genome Sequencing
Additional testing	None

SUMMARY OF RESULTS

Primary Findings

Variants related to the patient's phenotype: A pathogenic variant was found in *BRAF*.

Gene	Variant	Zygosity	Parental segregation	Disease, Mode of inheritance	Classification
BRAF	c.1914T>C (p.Asp638Glu)	Heterozygous	Unknown	*BRAF*-related disorders, Autosomal dominant	Pathogenic

Secondary findings

Pathogenic and likely pathogenic variants in medically actionable genes defined by the American College for Medical Genetics and Genomics that are unrelated to the patient's phenotype

Gene	Variant	Zygosity	Parental segregation	Disease, Inheritance	Classification
PMS2	c.2113G>A (p.Glu705Lys)	Heterozygous	Unknown	Lynch syndrome, Autosomal dominant	Pathogenic

WHAT DO THE RESULTS MEAN?

A pathogenic variant in *BRAF* and the proband's phenotype are consistent with cardiofaciocutaneous syndrome or CFCS. Additional variant information, testing details, and resources are available in the following pages.

RECOMMENDATIONS

Genetic counseling is recommended to discuss the implications of this test report. Treatment and management of CFCS-related manifestations involves a multidisciplinary team and should be discussed with the patient's clinical provider. In addition, individuals with pathogenic variants in *PMS2* have increased risks for colorectal, endometrial and other types of cancer. Carrier testing could be considered in family members at risk of having inherited the variant.

VARIANT DETAILS

Clinical Information

Candidate variants were interpreted in the context of the following clinical, phenotypic and medical history provided on the test requisition.

Phenotypes	Profound global developmental delay (HP:0012736); Seizure (HP:0001250); Cerebral visual impairment (HP:0100704); Hypertelorism (HP:0000316); Gastroesophageal reflux (HP:0002020); Stroke (HP:0001297); Hemiplegia (HP:0002301)

Primary Findings

BRAF c.1914T>G (p.Asp638Glu)

Gene	Genomic variant (GRCh38)	Transcript variant	Protein variant	Zygosity	Parental segregation
BRAF	NC_000007.14:g.140749365A>C	NM_004333.6:c.1914T>G	p.Asp638Glu	Heterozygous	Unknown

Gene function	*BRAF* encodes a serine/threonine protein kinase and effector of RAS signaling.
Disease association	Pathogenic variants in *BRAF* account for around 75% of cases of cardiofaciocutaneous syndrome (CFCS), an autosomal dominant disorder characterized by cardiac anomalies, distinctive facial features, and suggestive ectodermal findings (PMID: 20301365). CFCS has an overlapping clinical spectrum with other RASopathies, including Costello, LEOPARD and Noonan syndromes. Developmental delay, seizures, and gastroesophageal reflux show variable expressivity in CFCS.
Variant interpretation	Pathogenic: PS2, PS4, PS3_Supporting, PM2, PP3 ● This variant has been reported in multiple individuals with CFCS in the literature, and the variant has been confirmed *de novo* in several cases (PS2, PS4; PMID: 16804887, 18039235, 19206169, 21063443, 22495831, 34573299). ● The variant has reduced kinase activity compared to the wild type protein *in vitro* and is associated with developmental phenotypes in zebrafish (PS3_Supporting; PMID: 18413255, 19376813). ● It is not found in population databases (PM2). ● The Asp638Glu variant occurs in the protein kinase domain and is predicted to be damaging by multiple *in silico* tools (PP3).
CONCLUSION	*BRAF* c.1914T>G (p.Asp638Glu) is a known pathogenic variant. This finding and the proband's phenotype are consistent with CFCS.
RECOMMENDATIONS	● Site-specific testing of the variant could be considered in the proband's parents to determine whether this variant occurs *de novo*. Confirmation that the variant occurs *de novo* would not change the classification of this variant.

Secondary Findings

PMS2 c.2113G>A (p.Glu705Lys)

Gene	Genomic variant (GRCh38)	Transcript variant	Protein variant	Zygosity	Parental segregation
PMS2	NC_000007.14:g.5982885C>T	NM_000535.7:c.2113G>A	p.Glu705Lys	Heterozygous	Unknown

Gene function	*PMS2* encodes a member of the DNA mismatch repair (MMR) pathway involved in the recognition and repair of replication-associated single base mismatches and small insertions and deletions. *MLH1*, *MSH2*, *MSH6* and *PMS2* are classical tumour suppressor genes whose loss is associated with characteristic microsatellite instability in the tumour. PMS2 forms a heterodimer with MLH1, and biallelic loss of either gene is associated with loss of expression of the PMS2 protein by immunohistochemistry.
Disease association	*PMS2* encodes a member of the DNA mismatch repair (MMR) pathway involved in the recognition and repair of replication-associated single base mismatches and small insertions

	and deletions. *MLH1*, *MSH2*, *MSH6* and *PMS2* are classical tumour suppressor genes whose loss is associated with characteristic microsatellite instability in the tumour. PMS2 forms a heterodimer with MLH1, and biallelic loss of either gene is associated with loss of expression of the PMS2 protein by immunohistochemistry.
Variant interpretation	Pathogenic: PS4, PS3_Moderate, PM2 ● This variant has been reported in multiple families with Lynch syndrome-related cancers and in the compound heterozygous state in constitutional mismatch repair deficiency syndrome (PS4; PMID: 26110232, 17029773, 9419979, 16619239, 26318770, 18602922). ● The variant disrupts PMS2 function and inhibits MMR activity *in vitro* through a recessive rather than dominant negative manner and has been associated with protein deficiency and MSI in patient tumours (PS3_Moderate; PMID: 17029773, 24027009, 18768816, 20624957). ● It is found at a frequency of $2.6x10^{-5}$ (4 of 151,518 alleles) in the gnomAD population database (PM2).
CONCLUSION	*PMS2* c.2113G>A (p.Glu705Lys) is a known pathogenic variant in Lynch syndrome.
RECOMMENDATIONS	● Site-specific testing of the variant should be considered in family members at risk of having inherited the variant to assess their risk for cancer ● Guidelines for cancer screening should be discussed with the patient's family given their personal and family risk for cancer.

METHODS

Clinical whole genome sequencing was performed at Psomagen, a CAP-certified and CLIA-accredited laboratory, and bioinformatic analysis and clinical reporting performed at Breakthrough Genomics. Relevant disease, medical and phenotypic information provided by the health care provider on the test requisition form will be used to help prioritize and interpret variants that may best explain the patient's phenotype. Enliter™ aggregates information from multiple population, clinical, and functional prediction databases to facilitate variant interpretation. Pathogenic and likely pathogenic variants reported in HGMD® and ClinVar, as well as variants with a minor allele frequency of less than 1% in the gnomAD population database, are prioritized for review. All pertinent inheritance patterns are considered. Variants of potential relevance identified by NGS are individually validated for quality based on extensive and continuous validation processes. Variants which meet our internal QC criteria are not validated by Sanger sequencing.

Analysis pipeline details:

Reference assembly	GRCh37
Software	BWA (0.7.17-r1188), bedtools (2.17.0), bcftools (1.6), GATK (4.1.3), Picard (2.8.2-4-g2105a1e), samtools (1.6),
Databases (version)	Population: 1000 Genomes (Phase 3), dbSNP (153), ESP (2014-11-03), ExAC (r1), gnomAD (r2.1) Clinical: ClinVar (2022-07), OMIM (2022-03) Genes: Ensembl (2011-04), GENCODE (19), RefSeq (2015-01) Functional prediction: PolyPhen (2.2.2), SIFT (5.2.2)

VARIANT INTERPRETATION

Variant classification is performed according to American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology criteria. Small SNV/indels and copy number variants are evaluated with respect to their pathogenicity and causality and categorized into one of five categories: pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign and benign.

Primary findings: Primary findings are pathogenic, likely pathogenic and VUS that are related to the indication for testing. Primary findings are returned for two categories of variants:

1. Variants related to the patient's phenotype: variants in genes with known or putative associations with human disease that are consistent with the patient's phenotype.
2. Variants possibly related to the patient's phenotype: variants in genes with known or putative associations with human disease that do not fully explain the patient's phenotype based on available evidence.

Secondary findings: When requested, secondary findings in medically actionable genes that are unrelated to the primary indication for testing are reported according to ACMG v3.0 guidelines. Only pathogenic and likely pathogenic variants in 73 genes recommended for return of secondary findings will be reported.

For VUS that have a potential impact on gene expression, splicing and/or epigenetic regulation and may be related to the patient's phenotype, RNA sequencing and/or methylation array testing are used to generate clarifying or supporting evidence and are not yet accredited or licensed as independent diagnostic assays. If you have any questions about how additional testing is used to help interpret the results of genome sequencing, please contact Alamya Health (sales@alamyahealth.com).

LIMITATIONS

Absence of a pathogenic variant does not exclude a genetic basis for disease. Test results are interpreted in the context of clinical findings, family history, and other laboratory data. If the results of this test do not explain the clinical findings, additional testing and/or updating the phenotypic information provided should be considered. Certain types of disease-causing variation, including complex indels, single-exon deletions and duplications, copy number neutral SVs, and repeat expansions, may not be reliably detected by this test. In addition, variants in certain regions of the genome with low sequencing coverage or alignment quality may not be detected. Our bioinformatics analysis pipeline is optimized to reduce false positives in pseudogenic sequences or other highly-homologous genomic regions; however, these may result in false negative findings for both sequencing and deletion/duplication analysis and may miss true pathogenic variants.

ADDITIONAL INFORMATION

This test has been developed and validated for clinical purposes by Psomagen and Breakthrough Genomics. The US Food and Drug Administration (FDA) has determined that clearance or approval of this method is not necessary and thus neither have been obtained. To ensure sample identity, several guidelines recommend testing a second sample that is independently obtained from the proband. Please note that any further analysis will result in additional costs. The classification of variants can change over time. Please contact Breakthrough Genomics (info@btgenomics.com) to determine if there have been any changes in classification of any reported variants.

Wenhui Laura Li
Wenhui Laura Li, Ph.D., FACMGG.
Clinical Laboratory Director

YYYY-MM-DD
Date Signed

REFERENCES

1. Rauen, K. A. Cardiofaciocutaneous Syndrome. GeneReviews® (2016).
2. Rauen, K. A. Distinguishing Costello versus cardio-facio-cutaneous syndrome: BRAF mutations in patients with a Costello phenotype. Am. J. Med. Genet. Part A 140A, 1681–1683 (2006).
3. Yoon, G., Rosenberg, J., Blaser, S. & Rauen, K. A. Neurological complications of cardio-facio-cutaneous syndrome. Dev. Med. Child Neurol. 49, 894–899 (2007).
4. Sarkozy, A. et al. Germline BRAF mutations in noonan, LEOPARD, and cardiofaciocutaneous Syndromes: Molecular diversity and associated phenotypic spectrum. Hum. Mutat. 30, 695–702 (2009).
5. Kleefstra, T. et al. Mitochondrial dysfunction and organic aciduria in five patients carrying mutations in the Ras-MAPK pathway. Eur. J. Hum. Genet. 2011 192 19, 138–144 (2010).
6. Abe, Y. et al. Prevalence and clinical features of Costello syndrome and cardio-facio-cutaneous syndrome in Japan: Findings from a nationwide epidemiological survey. Am. J. Med. Genet. Part A 158A, 1083–1094 (2012).
7. Battaglia, D. I. et al. Epilepsy and braf mutations: Phenotypes, natural history and genotype-phenotype correlations. Genes (Basel). 12, (2021).
8. Rodriguez-Viciana, P. & Rauen, K. A. Biochemical Characterization of Novel Germline BRAF and MEK Mutations in Cardio-Facio-Cutaneous Syndrome. Methods Enzymol. 438, 277–289 (2008).
9. Anastasaki, C., Estep, A. L., Marais, R., Rauen, K. A. & Patton, E. E. Kinase-activating and kinase-impaired cardio-facio-cutaneous syndrome alleles have activity during zebrafish development and are sensitive to small molecule inhibitors. Hum. Mol. Genet. 18, 2543–2554 (2009).
10. Suerink, M. et al. The effect of genotypes and parent of origin on cancer risk and age of cancer development in PMS2 mutation carriers. Genet. Med. 18, 405–409 (2016).
11. Deschênes, S. M. et al. The E705K mutation in hPMS2 exerts recessive, not dominant, effects on mismatch repair. Cancer Lett. 249, 148–156 (2007).
12. Miyaki, M. et al. Drastic genetic instability of tumors and normal tissues in Turcot syndrome. Oncogene 1997 1523 15, 2877–2881 (1997).
13. Clendenning, M. et al. Long-range PCR facilitates the identification of PMS2-specific mutations. Hum. Mutat. 27, 490–495 (2006).
14. Lavoine, N. et al. Constitutional mismatch repair deficiency syndrome: clinical description in a French cohort. J. Med. Genet. 52, 770–778 (2015).
15. Senter, L. et al. The Clinical Phenotype of Lynch Syndrome Due to Germ-Line PMS2 Mutations. Gastroenterology 135, 419-428.e1 (2008).
16. Drost, M., Koppejan, H. & De Wind, N. Inactivation of DNA mismatch repair by variants of uncertain significance in the PMS2 gene. Hum. Mutat. 34, 1477–1480 (2013).
17. Marinovic-Terzic, I. et al. Apoptotic function of human PMS2 compromised by the nonsynonymous single-nucleotide polymorphic variant R20Q. Proc. Natl. Acad. Sci. U. S. A. 105, 13993–13998 (2008).
18. Van Oers, J. M. M. et al. PMS2 endonuclease activity has distinct biological functions and is essential for genome maintenance. Proc. Natl. Acad. Sci. U. S. A. 107, 13384–13389 (2010).