Cerebrum DX

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Scientific Director: George Nasioulas PhD

SAMPLE INFORMATION

Name :	-	**Date Received :**	-
Medical ID :	-	**Date of Report :**	-
Date of Birth :	-	**Req. Physician :**	-
Location :	-	**Barcode :**	-
Material :	WHOLE PERIPHERAL BLOOD	**Reason of referal:**	Myopathies

CerebrumDX analysis by Next Generation Sequencing

Results associated with the reason of referral

Gene	Variant	Clinical Significance	Zygosity
COL4A2	NM_001846.4:c.964C>T, p.(Arg322*)	Likely Pathogenic variant	Heterozygous
PLEC	NM_201378.4:c.3485G>A, p.(Arg1162His)	Variant of Uncertain Significance (VUS)	Heterozygous

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Variants Details

***COL4A2*, Exon 17, NM_001846.4:c.964C>T, p.(Arg322*)** ClinGen HPO ClinVar

This sequence change creates a premature translational stop signal (p.Arg322*) in the *COL4A2* gene. It is expected to result in an absent or disrupted protein product. Loss-of-function variants in COL4A2 are known to be pathogenic (PMID: 22333902, 30315939). This variant is present in population databases at a very low frequency (rs1237717942, gnomAD_exom <0.01%) but is not listed in the mutation database ClinVar. Based on the classification criteria set by the ACMG and AMP (PMID: 25741868) this variant has been classified as likely pathogenic. According to international guidelines it is recommended that relatives of the patient are tested for the above mutation.

The *COL4A2* gene has been localized to chromosome 13q34 and encodes the alpha-2 chain of type IV collagen. Type IV collagen is associated with laminin, entactin and heparan sulfate proteoglycans to form the sheetlike basement membranes that separate epithelium from connective tissue. *COL4A2* mutations are a novel major risk factor for familial cerebrovascular disease, including porencephaly and small-vessel disease with reduced penetrance and variable phenotype, which might also be modified by other contributing factors. Additionally, the *COL4A2* gene has preliminary evidence supporting a correlation with autosomal recessive leukoencephalopathy (PMID: 36324412, 22333902, 33912663, 36603335).

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PLEC **,Exon 27, NM_201378.4:c.3485G>A, p.(Arg1162His)** ClinGen HPO ClinVar

Gene	***PLEC***	OMIM
Disease associated with pathogenic variants in this gene	Epidermolysis bullosa simplex 5A, Ogna type, Autosomal dominantpidermolysis bullosa simplex 5B, with muscular dystrophy,Autosomal recessive	
Variant	NM_201378.4:c.3485G>A, p.(Arg1162His)	
Zygosity	Heterozygous	
Type of variant	Missense	
Allele frequency (dbSNP)	0.01%	rs782533009
Grantham score	29	
Protein position information	In a domain of the protein that is not known to be functionally important	
ClinVar	Variation ID: 851190	
In silico analysis	Tolerated	
Clinical Significance	Variant of Uncertain Significance (VUS)	

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Methodology

Genomic DNA was extracted from the sample under investigation and was analysed by a solution based capture approach using the target enrichment panel (KAPA HyperCap Heredity Panel, Roche). Sequencing was carried out using the DNBSEQ-G400 sequencing platform (MGI). Reads were aligned to the reference sequence (GRCh37), and sequence changes were identified and interpreted in the context of a single clinically relevant transcript. Unless otherwise stated, this assay targets all coding regions of the indicated transcripts and 10 base pairs of flanking intronic sequences. The mean coverage was …x with ..% of all targeted regions sequenced with >=20x depth.

Based on the available patient information, the following diagnostic algorithm was used.

- The genes described in the OMIM and HPO databases were selected as genes associated with the patient phenotype.
- Variant classification was performed according to the ACMG AND AMP guidelines (PMID: 25741868)
- Analysis of the mutations with damaging effect (frameshift, nonsense, missense, splicing mutations etc.) as well as de novo mutations was obtained.
- All clinically significant observations were confirmed by Sanger Sequencing analysis on SeqStudio Genetic Analyzer (Thermo Fisher Scientific).

*Note:

Every molecular test has an internal 0,5-1% chance of failure. This is due to rare molecular events and factors related to the preparation and analysis of the samples. Unless otherwise stated, this assay targets all coding regions of the indicated transcripts and 10 base pairs of flanking intronic sequences. Therefore, this method cannot detect variants in deep intronic or enhancer/promoter regions.

The method used achieves 99% sensitivity and specificity for single nucleotide variants and insertions and deletions

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Details about non-pathogenic variants

All individuals carry DNA changes (i.e., variants), and most variants do not increase an individual's risk of the disease. When identified, variants of uncertain significance (VUS) are reported. Benign variants (Polymorphisms) are not reported and available data indicate that these variants most likely do not cause increased risk for the disease. Present evidence does not suggest that non-clinically significant variant findings be used to modify patient medical management beyond what is indicated by the personal and family history and any other clinically significant findings.

Genes Analyzed (Table 1)

ACTA1	*ANO5*	*ATP2A1*	*B3GALNT2*	*BAG3*	*BICD2*	*BIN1*	*CAPN3*	*CAV3*
CFL2	*CHKB*	*COL12A1*	*COL4A1*	*COL4A2*	*COL6A1*	*COL6A2*	*COL6A3*	*CRPPA*
CRYAB	*DAG1*	*DES*	*DMD*	*DNAJB6*	*DYSF*	*EMD*	*FDX2*	*FHL1*
FKRP	*FKTN*	*FLNC*	*GAA*	*GMPPB*	*GOSR2*	*HNRNPDL*	*INPP5K*	*ITGA7*
KBTBD13	*KLHL40*	*KLHL41*	*LAMA2*	*LARGE1*	*LDB3*	*LMNA*	*LMOD3*	*MEGF10*
MICU1	*MME*	*MSTO1*	*MTM1*	*MYH7*	*MYO18B*	*MYOT*	*NEB*	*PLEC*
PNPLA2	*POGLUT1*	*POLG*	*POMGNT1*	*POMGNT2*	*POMK*	*POMT1*	*POMT2*	*PYROXD1*
RBCK1	*RYR1*	*SELENON*	*SEPTIN9*	*SGCA*	*SGCB*	*SGCD*	*SGCG*	*SMCHD1*
SPEG	*SPTBN4*	*SYNE1*	*TCAP*	*TK2*	*TMEM126B*	*TMEM43*	*TNNT1*	*TNPO3*
TPM2	*TPM3*	*TRAPPC11*	*TRIM32*	*TTN*	*VMA21*	*VPS13A*		

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Family tree

Note: **The information shown on the family tree has been provided by the patient and not by medical records.**

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Literature

1. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. **Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.** Genet Med. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30. Epub 2015 Mar 5. (PMID: 25741868) PMCID: PMC4544753.
2. Harrison SM, Biesecker LG, Rehm HL. **Overview of Specifications to the ACMG/AMP Variant Interpretation Guidelines.** Curr Protoc Hum Genet. 2019 Sep;103(1):e93. doi: 10.1002/cphg.93. (PMID: 31479589) PMCID: PMC6885382.
3. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, Herman GE, Hufnagel SB, Klein TE, Korf BR, McKelvey KD, Ormond KE, Richards CS, Vlangos CN, Watson M, Martin CL, Miller DT. **Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics.** Genet Med. 2017 Feb;19(2):249-255. doi: 10.1038/gim.2016.190. Epub 2016 Nov 17. Erratum in: Genet Med. 2017 Apr;19(4):484. PMID: 27854360.
4. Landrum MJ, Chitipiralla S, Brown GR, Chen C, Gu B, Hart J, Hoffman D, Jang W, Kaur K, Liu C, Lyoshin V, Maddipatla Z, Maiti R, Mitchell J, O Leary N, Riley GR, Shi W, Zhou G, Schneider V, Maglott D, Holmes JB, Kattman BL. **ClinVar: improvements to accessing data.** Nucleic Acids Res. 2020 Jan 8;48(D1):D835-D844. doi: 10.1093/nar/gkz972. (PMID: 31777943) PMCID: PMC6943040.
5. Köhler S, Gargano M, Matentzoglu N, Carmody LC, Lewis-Smith D, Vasilevsky NA, Danis D, Balagura G, Baynam G, Brower AM, Callahan TJ, Chute CG, Est JL, Galer PD, Ganesan S, Griese M, Haimel M, Pazmandi J, Hanauer M, Harris NL, Hartnett MJ, Hastreiter M, Hauck F, He Y, Jeske T, Kearney H, Kindle G, Klein C, Knoflach K, Krause R, Lagorce D, McMurry JA, Miller JA, Munoz-Torres MC, Peters RL, Rapp CK, Rath AM, Rind SA, Rosenberg AZ, Segal MM, Seidel MG, Smedley D, Talmy T, Thomas Y, Wiafe SA, Xian J, Yüksel Z, Helbig I, Mungall CJ, Haendel MA, Robinson PN. **The Human Phenotype Ontology in 2021.** Nucleic Acids Res. 2021 Jan 8;49(D1):D1207-D1217. doi: 10.1093/nar/gkaa1043. (PMID: 33264411) PMCID: PMC7778952.
6. Rivera-Muñoz EA, Milko LV, Harrison SM, Azzariti DR, Kurtz CL, Lee K, Mester JL, Weaver MA, Currey E, Craigen W, Eng C, Funke B, Hegde M, Hershberger RE, Mao R, Steiner RD, Vincent LM, Martin CL, Plon SE, Ramos E, Rehm HL, Watson M, Berg JS. **ClinGen Variant Curation Expert Panel experiences and standardized processes for disease and gene-level specification of the ACMG/AMP guidelines for sequence variant interpretation.** Hum Mutat. 2018 Nov;39(11):1614-1622. doi: 10.1002/humu.23645. (PMID: 30311389) PMCID: PMC6225902.
7. Miller DT, Lee K, Gordon AS, Amendola LM, Adelman K, Bale SJ, Chung WK, Gollob MH, Harrison SM, Herman GE, Hershberger RE, Klein TE, McKelvey K, Richards CS, Vlangos CN, Stewart DR, Watson MS, Martin CL; ACMG Secondary Findings Working Group. **Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical Genetics and Genomics (ACMG).** Genet Med. 2021 May 20. doi: 10.1038/s41436-021-01171-4. Epub ahead of print. (PMID: 34012069)
8. Miller DT, Lee K, Chung WK, Gordon AS, Herman GE, Klein TE, Stewart DR, Amendola LM, Adelman K, Bale SJ, Gollob MH, Harrison SM, Hershberger RE, McKelvey K, Richards CS, Vlangos CN, Watson MS, Martin CL; ACMG Secondary Findings Working Group. **ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG).** Genet Med. 2021 May 20. doi: 10.1038/s41436-021-01172-3. Epub ahead of print. (PMID: 34012068)

7. Verbeek E et al. **COL4A2 mutation associated with familial porencephaly and small-vessel disease.** Eur J Hum Genet. 2012 Aug;20(8):844-51. doi: 10.1038/ejhg.2012.20. (PMID: 22333902)

8. Nicita F et al. **Leukoencephalopathy with spot-like calcifications caused by recessive COL4A2 variants.** Clin Neurol Neurosurg. 2023 Feb;225:107584. doi: 10.1016/j.clineuro.2022.107584. (PMID: 36603335)

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9. Guey S et al. **Main features of COL4A1-COL4A2 related cerebral microangiopathies.** Cereb Circ Cogn Behav. 2022 Mar 24;3:100140. doi: 10.1016/j.cccb.2022.100140. (PMID: 36324412)
10. Richards S et al. **Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics** Genet Med. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30. (PMID: 25741868)
11. Bakhtiari S et al. **Recessive COL4A2 Mutation Leads to Intellectual Disability, Epilepsy, and Spastic Cerebral Palsy.** Neurol Genet. 2021 Apr 22;7(3):e583. doi: 10.1212/NXG.0000000000000583. (PMID: 33912663)
12. Cavallin M et al. **Further refinement of COL4A1 and COL4A2 related cortical malformations.** Eur J Med Genet. 2018 Dec;61(12):765-772. doi: 10.1016/j.ejmg.2018.10.004. (PMID: 30315939)