XXX

Order no.: xxx
Order received: xxx
Sample type: blood, EDTA
Sample collection date: xxx
Report type: Final Report
Report date: xxx

Patient no.: **xxx**, First Name: **xxx**, Last Name: **xxx**
DOB: **xxx**, Sex: **xxx**, Your ref.: **xxx**

Test(s) requested: Neuronal migration disorders panel (sequencing, deletion/duplication analysis)

CLINICAL INFORMATION

Patient with epilepsy, developmental delay, subcortical band heterotopia.

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A heterozygous pathogenic variant was identified in the DCX gene. **The genetic diagnosis of subcortical band heterotopia is confirmed.**

In the remainder of the panel genes (see methods), no other clinically relevant variant has been identified by sequencing and MLPA.

RECOMMENDATIONS

- Parental carrier testing is requested to establish whether the detected variant in the DCX gene is inherited or de novo.
- Genetic counselling is recommended.

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RESULT SUMMARY

GENE	VARIANT COORDINATES	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
DCX	ChrX(GRCh37):g.110574171G>A NM_000555.3:c.1150C>T p.(Arg384*) Exon 5	Het	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation: nt weak	gnomAD: - ESP: - 1000 G: - CentoMD: -	Stop gain Pathogenic (class 1)

Variant description based on Alamut Batch (latest database available). * AlignGVD: C0: least likely to interfere with function, C65: most likely to interfere with function, splice prediction tools: SSF, MaxEnt, HSF. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD® (latest database available). *** based on ACMG recommendations

VARIANT INTERPRETATION

DCX, c.1150C>T p.(Arg384*)

The DCX variant c.1150C>T p.(Arg384*) creates a premature stop codon. According to HGMD Professional 2017.3, this variant has previously been described as disease causing for Double cortex syndrome by des Portes et al., 1998 (PMID: 9618162), Kato et al., 1999 (PMID: 10369164), Xiong et al., 2015 (PMID: 25525159). ClinVar lists this variant as pathogenic (clinical testing, Variation ID: 158511). It is classified as pathogenic (class 1) according to the recommendations of Centogene and ACMG (please, see additional information below).

DCX-related disorders include the neuronal migration disorders classic lissencephaly (formerly also known as lissencephaly type 1), usually in males; and subcortical band heterotopia (SBH, also called double cortex), primarily in females. In individuals with SBH, cognitive abilities range from normal to learning disabilities and/or severe intellectual disability. The majority of individuals with SBH present with focal or generalized seizures. Behavior problems may also be observed. In DCX-related lissencephaly and SBH the severity of the clinical manifestation correlates with the degree of the underlying brain malformation. DCX-related disorders are inherited in an X-linked manner. Approximately 10% of unaffected mothers of children with a DCX pathogenic variant were reported to have somatic mosaicism or germline mosaicism. Heterozygous females may be asymptomatic pathogenic variant carriers or more frequently manifest a wide phenotypic spectrum of SBH (Hehr et al., 2011 - PMID: 20301364).

CENTOGENE VARIANT CLASSIFICATION (BASED ON ACMG RECOMMENDATIONS)

Class 1 – Pathogenic
Class 2 – Likely pathogenic
Class 3 – Variant of uncertain significance (VUS)
Class 4 – Likely benign
Class 5 – Benign
Additionally, other types of clinical relevant variants can be identified (e.g. risk factors, modifiers).

METHODS

Genomic DNA is enzymatically fragmented and regions of interest are selectively enriched using capture probes targeted against coding regions of panel genes. Libraries are generated with Illumina compatible adaptors and sequenced on an Illumina platform.
For the Neuronal migration disorders panel, the entire coding region of the ACTB, ACTG1, ADGRG1, ARFGEF2, ARX, COL18A1, COL4A1, CPT2, DCX, EMX2, EOMES, FGFR3, FH, FKRP, FKTN, FLNA, IER3IP1, ISPD, LAMA2, LAMC3, LARGE, MED12, MEF2C, OCLN, PAFAH1B1, PAX6, PEX7, POMGNT1, POMT1, POMT2, PQBP1, RAB18, RAB3GAP1, RAB3GAP2, RELN, SNAP29, SRPX2, TUBA1A, TUBA8, TUBB2B, TUBB3, VDAC1, WDR62 genes including 10 bp of flanking intronic sequences are targeted. Our Plus Panel includes analysis of all reported disease causing deep intronic and regulatory mutations described outside the coding +/-10 boundary. Due to limitations of the method, the targeted sequences within the requested panel may not be covered 100%. Missing regions or regions of poor quality are completed with classical Sanger sequencing to achieve 100% coverage of all genes within this panel. Raw sequence data analysis, including base calling, demultiplexing, alignment to the hg19 human reference genome (Genome Reference Consortium GRCh37) and variant calling is performed using validated in-house software. All identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized into classes 1 - 5 (see above). All variants related to the phenotype of the patient, except benign or likely benign variants, are

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reported. Structural changes (i.e. CNVs, inversions, repeat expansions etc.) are not assessed with the NGS data.

MLPA (multiplex ligation-dependent probe amplification) analyses were performed using SALSA MLPA probemix P061-D1, P061-D1/qPCR, P080-C1, P116-B1, P189-C2, P198-A3, P219-B3, P259-B2, P326-A2, P391-A2/P392-A2, P395-A2 provided by MRC-Holland to test for deletions or duplications within or including the ARX, DCX, FGFR3, FH, FKRP, FKTN, FLNA, LAMA2, LARGE, MEF2C, PAFAH1B1, PAX6, POMGNT1, POMT1, POMT2, PQBP1 gene(s).

ADDITIONAL INFORMATION

This test was developed and its performance validated by CENTOGENE AG. 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. This test has been developed for clinical purposes. All test results are reviewed, interpreted and reported by our scientific and medical experts.

To also exclude mistaken identity in your clinic, 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 the time. Please feel free to contact CENTOGENE (dmqc@centogene.com) in the future to determine if there have been any changes in classification of any reported variants.

DISCLAIMER

Any preparation and processing of a sample from patient material provided to CENTOGENE by a physician, clinical institute or a laboratory (by a "Partner") and the requested genetic and/or biochemical testing itself is based on the highest and most current scientific and analytical standards. However, in very few cases genetic or biochemical tests may not show the correct result, e.g. because of the quality of the material provided by a Partner to CENTOGENE or in cases where any test provided by CENTOGENE fails for unforeseeable or unknown reasons that cannot be influenced by CENTOGENE in advance. In such cases, CENTOGENE shall not be responsible and/or liable for the incomplete, potentially misleading or even wrong result of any testing if such issue could not be recognized by CENTOGENE in advance.

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