CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE AG Am Strande 7 • 18055 Rostock • Germany

Dr.

Country

Order no.: XXXXXXX
Order received:
Sample type: blood, filter card
Sample collection date:
Report date:
Report type: Final Report

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

Test(s) requested: CentoLCV™ - Whole Genome NGS-based Large Copy number Variation analysis

CLINICAL INFORMATION

Abnormal facial shape; Abnormality of cardiovascular system morphology; Congenital onset; Delayed speech and language development; Failure to thrive; Generalized-onset seizure; Global developmental delay; Hypospadias; Intellectual disability; Intrauterine growth retardation; Motor delay
(Clinical information indicated above follows HPO nomenclature.)

Family history: Unknown.
Siblings unaffected.
Consanguineous parents: No.

The patient has a normal karyotype, but clinician suspects chromosome 4p- syndrome (Wolf-Hirschhorn syndrome).

POSITIVE RESULT
Chromosomal alteration identified

INTERPRETATION

We detected a 9.1 Mb large chromosome 4p terminal deletion (partial monosomy) and a 7.1 Mb large chromosome 8p terminal gain (partial trisomy). The detected genetic sex is: male.

The results are compatible with an unbalanced translocation between chromosomes 4 and 8, with breakpoints in cytobands 4p16.1 and 8p23.1, and with the genetic diagnoses of Wolf-Hirschhorn syndrome (4p-) and 8p23.1 duplication syndrome.

RECOMMENDATIONS

- Clinical correlation is recommended. Sharing these results with the cytogenetics laboratory, for karyotype reevaluation, is advised.
- Since CentoLCV™ does not detect balanced chromosomal rearrangements, we recommend cytogenetic analysis of both parents –karyotype and/or metaphase FISH- in order to investigate the origin of the unbalanced anomaly in the patient, *de novo* or arising from a balanced translocation in one of the parents. Please be informed that we do not offer this specific test.
- Genetic counselling and appropriate clinical management are recommended.

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RESULT SUMMARY

CNV DESCRIPTION*	SIZE (KB)	GENE COUNT**	INTERPRETATION***	PATIENT RELEVANT PHENOTYPE
seq[GRCh37] del(chr4)(p16.3p16.1) chr4:g.69001_9216000del	**9147**	**33**	**Pathogenic**	**Wolf-Hirschhorn syndrome (4p-)**
seq[GRCh37] dup(chr8)(p23.3p23.1) chr8:g.135001_7263000dup	**7128**	**5**		**8p23.1 duplication syndrome**

* according to ISCN 2016; ** genes are listed at the end of the report; *** according to ACMG 2011, modified

RESULTS INTERPRETATION

We detected a 9.1 Mb large chromosome 4p terminal deletion (partial monosomy) and a 7.1 Mb large chromosome 8p terminal gain (partial trisomy). Please note that the exact breakpoints cannot be determined by this method. The findings are compatible with an unbalanced translocation between chromosomes 4 and 8, with breakpoints in cytobands 4p16.1 and 8p23.1, and with the genetic diagnoses of Wolf-Hirschhorn syndrome (4p-) and 8p23.1 duplication syndrome. An inherited origin from a balanced chromosomal translocation in one of the parents is likely and therefore, parental chromosomal testing is warranted.

Wolf-Hirschhorn syndrome (OMIM®: 194190) is a developmental disorder characterized by typical craniofacial features, prenatal and postnatal growth impairment, intellectual disability, severe delayed psychomotor development, seizures, and hypotonia. Marked intra-uterine growth retardation and ongoing postnatal slow weight gain are observed. Patients have a distinctive facies characterized by the "Greek warrior helmet" appearance (broad nasal bridge continuing to the forehead), visible more clearly before puberty, microcephaly, high forehead with prominent glabella, hypertelorism, epicanthus, highly arched eyebrows, short filtrum, downturned mouth, micrognathia, poorly formed ears with pits/tags and, in some cases, cleft lip/palate. Skeletal anomalies include kyphosis or scoliosis with malformed vertebral bodies, accessory or fused ribs, clubfeet and split hand. Patients suffer from hypotonia with muscle underdevelopment, possibly causing frequent feeding difficulties and may lead to failure to thrive. Developmental delay is severe. Intellectual deficit is moderate to severe, rarely mild. Speech is limited to guttural or disyllabic sounds, except in a few patients achieving formulation of simple sentences. Various types of seizures are observed in up to 95%, onset is between the neonatal period and 36 months and the triggering factor is often fever. Status epilepticus occurs in half of patients. Over 30% of children develop atypical absences by age 1 to 6. In approximately 50%, seizures stop in childhood. Most patients have structural central nervous system defects, mainly including thinning of the corpus callosum. Congenital heart defects, and mostly atrial septal defect, are seen in 50%. Ophtalmologic, auditory and dental anomalies are also frequently observed. Patients may have recurrent respiratory tract infections and otitis media, due to antibodies deficiency (Ig1 or IgG2 subclass). Urinary tract malformations have been described, and half of male patients have hypospadias and cryptorchidism. Treatment is symptomatic and requires multidisciplinary management including diverse rehabilitation programs, seizure treatment (valproic acid with or without ethosuccimide) and feeding therapies (ORPHA: 280).

8p23.1 duplication syndrome is a rare chromosomal anomaly syndrome, resulting from the partial duplication of the short arm of chromosome 8, with a highly variable phenotype, principally characterized by mild to moderate developmental delay, intellectual disability, mild facial dysmorphism (incl. prominent forehead, arched eyebrows, broad nasal bridge, upturned nares, cleft lip and/or palate) and congenital cardiac anomalies (e.g., atrioventricular septal defect). Other reported features include macrocephaly, behavioral abnormalities (e.g., attention deficit disorder), seizures, hypotonia and ocular and digital anomalies (poly/syndactyly) (ORPHA: 251076).

Please note that the result of the chromosomal rearrangement may not reflect the phenotype associated with each of the alterations described separately, but a different phenotype resulting from the interaction of both in the genomic context of the patient.

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GENES INCLUDED IN THE DETECTED CNVs:

CNV DESCRIPTION*	Protein-coding RefSeq GENES**
seq[GRCh37] del(chr4)(p16.3p16.1) chr4:g.69001_9216000del	*ZNF595, ZNF141, PIGG, PDE6B, SLC26A1, IDUA, RNF212, CTBP1, UVSSA, TACC3, FGFR3, LETM1, NSD2, NELFA, NAT8L, POLN, HAUS3, SH3BP2, ADD1, GRK4, HTT, RGS12, DOK7, LRPAP1, ADRA2C, MSX1, STK32B, EVC2, EVC, WFS1, PPP2R2C, TRMT44, HMX1*
seq[GRCh37] dup(chr8)(p23.3p23.1) chr8:g.135001_7263000dup	*DLGAP2, CLN8, ARHGEF10, MCPH1, ANGPT2*

* according to ISCN 2016; ** according to NCBI

ANALYSIS STATISTICS

The target sequence was covered >3x (average coverage was 5.4x).

CENTOGENE CLASSIFICATION OF DETECTED COPY NUMBER VARIANTS

PATHOGENIC – CNV with sufficient evidence to classify as pathogenic
LIKELY PATHOGENIC – CNV with strong evidence in favor of pathogenicity
UNCERTAIN SIGNIFICANCE – CNV with limiting and/or conflicting evidence regarding pathogenicity
LIKELY BENIGN – CNV with strong evidence against pathogenicity
BENIGN – CNV with sufficient evidence to classify as benign; polymorphism

Additionally, other types of clinical relevant variants can be identified (e.g. risk factors, modifiers).

The classification of copy number variants at CENTOGENE is based on the ACMG standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants (2011). Copy number variants are evaluated based on the patient's reason for referral for this genomic screening. Comprehensive reporting of heterozygous recessive variants is outside the scope of the intended use of this test. Therefore, recessive carrier status might not be disclosed. Any clinical concern for recessive disorders should be communicated to the reporting laboratory for appropriate consideration.

1 copy loss – heterozygous/hemizygous deletion
2 copy loss – homozygous deletion
1 copy gain – heterozygous/hemizygous gain
2 copy gain – homozygous gain

METHODS

Genomic DNA is enzymatically fragmented and libraries are generated by PCR-mediated addition of Illumina compatible adaptors. Final libraries are sequenced at 2 x 150 bp paired-end ~500bp insert sizes on an Illumina platform (HiSeq X or HiSeq 4000) with a mean coverage of >3x. Alignment to the human reference genome (GRCh37/hg19) and CNV calling is performed based on an internal pipeline including multiple callers. Bioinformatic analysis is restricted to potentially clinically relevant genomic regions encompassing the complete nuclear genome. Interrogated regions do not include highly repetitive DNA sequences, such as the short arm of acrocentric chromosomes, centromeres, telomeres and other heterochromatin blocks. Copy number variations with a minimum size of 50 kb for deletions and 200kb for duplications are analyzed. A lower size threshold is considered for homo/hemizygous deletions. The provided clinical information and family history and the current knowledge of genes and alterations at the time of reporting are used to evaluate the identified copy number variants in respect to their pathogenicity and causality which leads to classification into 5 classes (see above). All variants related to the phenotype of the patient, except benign or likely benign variants, are reported. CENTOGENE has established stringent quality criteria and validation processes for variants detected by shallow WGS. Lower quality aberrations are confirmed by MLPA, qPCR or microarray for reported variants.

LIMITATIONS

CentoLCV™ method is recommended for the purpose of identifying DNA copy number variations (CNVs) associated with large chromosomal imbalances, microdeletion/duplication syndromes, and partial or complete gene deletions/duplications based on shallow whole genome sequencing. CentoLCV™ can only detect large genomic copy number imbalances in the nuclear genome. It cannot detect balanced chromosomal rearrangements such as translocations and inversions, imbalances in the mitochondrial genome, repeat sequences such as segmental duplications or repeat expansions, regions with absence of heterozygosity, uniparental disomy, point mutations and delins, chromosomal mosaicism, sample contamination, complete ploidy changes, methylation aberrations, or copy number changes in the regions of the genome that are not targeted/covered. CentoLCV™ may not reliably detect CNV within or encompassing the pseudoautosomal regions of the sex chromosomes. Failure to detect an alteration at a specific locus does not exclude the diagnosis of a genetic disorder associated with that locus. There might be abnormalities present in that region that are not detectable by the CentoLCV™ technology.

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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 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 (customer.support@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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