NEURAZON
PRECISION HEALTH

Prof. Noraldin Al-Deri
Early Intervention Center for Autism and Developmental Disorders
510, 224 wasfi al tal
11953 Amman
Jordan

Case no: **1889285**
First Name: **Rayyan**
Last Name: **Nassar**
DOB: **18 Jun. 2020**
Sex: **M**
Your ref.: **1889285**

Test requested: Precision Health Analysis®

RESULTS: Acquired ASD and White Matter Delays Causes Detected

Potentially relevant finding identified

INTERPRETATION RECOMMENDATIONS

- Clinically relevant variants related to the described phenotype were detected.
- Please also note variant listed in the attached report.
- Reevaluation of the sequence dataset is recommended every 12 months or if there are phenotypic changes.
- Reassessment every 3 months by a multidisciplinary team to assess for any phenotypic changes observed.
- Tailored intervention plan is recommended under supervision of a multidisciplinary team.

ADDITIONAL INFORMATION

This assessment was developed, and its performance was validated, by the Ai model of NEURAZON and according to the findings in the report attached. 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. Please refer to attached report for details.

Prof. Noraldin Al-Deri
Early Intervention Center for Autism and Developmental Disorders
Diagnosis
510, 224 wasfi al tal
11953 Amman
Jordan

Order no.: 63197275

Report type: Final report

Patient no: **1889285**, First Name: **Rayyan**, Last Name: **Nassar**
DOB: **18 Jun. 2020**, Sex: **male**, Your ref.: **JWES**

Test(s) requested: CentoXome® Solo

CLINICAL INFORMATION

Attention deficit hyperactivity disorder; Autism; Autism with high cognitive abilities; Autistic behavior; Delayed speech and language development; Global developmental delay; Hyperactivity; Intellectual disability; Neurodevelopmental abnormality; Neurodevelopmental delay
(Clinical information indicated above follows HPO nomenclature.)
Family history: Unknown.
Consanguineous parents: Unknown.

RESULT

Potentially relevant finding identified

INTERPRETATION

Clinically relevant variants related to the described phenotype were detected.

Please also note the variant listed in the Potentially relevant findings section.

RECOMMENDATIONS

- Reevaluation of the sequence dataset is recommended every 12 months or if there are phenotypic changes. Additionally, proceeding to genome sequencing should be considered, given that up to 30% of cases with a negative exome sequencing result can be diagnosed by genome sequencing.
- Genetic counselling is recommended.

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POTENTIALLY RELEVANT FINDINGS

In this table we list variants related to disorders without an apparent overlap with the described phenotype of the patient and/or variants with a zygosity inconsistent with the expected mode of inheritance. As examples, a variant of uncertain significance (VUS) in a gene with only partial clinical overlap, or a single heterozygous pathogenic variant in a gene with a recessive phenotype which has clinical overlap, may be reported here. These variants are mentioned in this report due to the potential contribution to the phenotype of the patient and may help close possible diagnostic gaps. For variants that may be considered clinically relevant, clinical re-evaluation and/or further testing (e.g. familial segregation analysis) could clarify their contribution to patient's phenotype.

SEQUENCE VARIANTS

GENE	VARIANT COORDINATES	AMINO ACID CHANGE	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES **	TYPE AND CLASSIFICATION ***	RELATED DISORDER (OMIM®) AND MODE OF INHERITANCE
ACTL6B	NM_016188.4:c.185G>A	p.(Gly62Glu)	Heterozygous	PolyPhen: Benign Align-GVDG: N/A SIFT: Tolerated MutationTaster: Disease causing Conservation_nt: moderate Conservation_aa:	gnomAD: - ESP: - 1000G: - CentoMD: -	Missense Uncertain significance (class 3)	Intellectual developmental disorder with severe speech and ASD (618470), AD

Variant annotation based on CentoCloud Bioinformatics pipeline. * AlignGVD: C0: least likely to interfere with function, C65: most likely to interfere with function; splicing predictions: Ada and RF scores. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD® (latest database available). *** based on ACMG recommendations.

RESEARCH FINDINGS

Research variants (with potential relevance to the described phenotype) are variants in genes with no or only partial experimental evidence for their involvement in human disease.

The data was analyzed focusing on variants affecting protein function (nonsense, frameshift, conserved splice site and missense with high pathogenicity predictions) in genes with supporting evidence on zygosity, segregation or functional importance of the gene. Available literature or experimental data on expression and/or animal models were considered. However, no such variants could be identified for the patient.

SECONDARY FINDINGS

If consent is provided, in line with ACMG recommendations (ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing; Genetics in Medicine, 2023; PMID: 37347242) we report secondary findings, i.e. relevant pathogenic and likely pathogenic variants in the recommended genes for the indicated phenotypes in this publication.

We did not detect any relevant variants in the genes for which secondary findings are reported.

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CARRIERSHIP FINDINGS

In this table we list sequence variants previously ascertained or evaluated and classified in CENTOGENE as "pathogenic" and "likely pathogenic", in selected genes associated with recessive severe and early-onset Mendelian diseases. As only in-house classified variants are presented, it should not be considered a comprehensive list of variants in these genes and does not provide a complete list of potentially relevant genetic variants in the patient. The complete gene list can be found at www.centogene.com/carriership-findings (please contact CENTOGENE customer support if the gene list has been updated after this report was issued). Orthogonal validation was not performed for these variants. Therefore, if any variant is used for clinical management of the patient, confirmation by another method needs to be considered. Furthermore, the classification of these variants may change over time, however reclassification reports for these variants will not be issued. CENTOGENE is not liable for any missing variant in this list and/or any provided classification of the variants at a certain point of time. As the identified variants may indicate (additional) genetic risks or diagnoses in the patient and/or family and/or inform about reproductive risks, we recommend discussing these findings in the context of genetic counselling.

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SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION ***
AGXT	NM_000030.2:c.322T>C	p.(Trp108Arg)	rs180177197	Heterozygous	PolyPhen: Probably damaging Align-GVDG: N/A SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: high Conservation_aa:	gnomAD: 0.0000040 ESP: - 1000 G: - CentoMD: -	Missense Pathogenic (class 1)
CFTR	NM_000492.3:c.254G>A	p.(Gly85Glu)	rs75961395	Heterozygous	PolyPhen: Probably damaging Align-GVDG: N/A SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: high Conservation_aa:	gnomAD: 0.000040 ESP: - 1000 G: - CentoMD: -	Missense Pathogenic (class 1)
FAH	NM_000137.2:c.1062+5G>A	p.(?)	rs80338901	Heterozygous	PolyPhen: N/A Align-GVDG: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: Conservation_aa: 2/2 likely splice effect	gnomAD: 0.00037 ESP: - 1000 G: - CentoMD: -	Splicing Pathogenic (class 1)
GNRHR	NM_000406.2:c.317A>G	p.(Gln106Arg)	rs104893836	Heterozygous	PolyPhen: Probably damaging Align-GVDG: N/A SIFT: - MutationTaster: Disease causing Conservation_nt: high Conservation_aa:	gnomAD: 0.0028 ESP: - 1000 G: - CentoMD: -	Missense Pathogenic (class 1)

Variant annotation based on CentoCloud Bioinformatics pipeline. * AlignGVD: C0: least likely to interfere with function, C65: most likely to interfere with function; splicing predictions: Ada and RF scores. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD® (latest database available). *** based on ACMG recommendations.

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 clinically relevant variants can be identified (e.g. risk factors, modifiers).

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METHODS

CentoXome® Solo

Genomic DNA is enzymatically fragmented, and target regions are enriched using DNA capture probes. These regions include approximately 41 Mb of the human coding exome (targeting > 98% of the coding RefSeq from the human genome build GRCh37/hg19), as well as the mitochondrial genome. The generated library is sequenced on an Illumina platform to obtain at least 20x coverage depth for > 98% of the targeted bases. An in-house bioinformatics pipeline, including read alignment to GRCh37/hg19 genome assembly and revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (NC_012920), variant calling, annotation, and comprehensive variant filtering is applied. All variants with minor allele frequency (MAF) of less than 1% in gnomAD database, and disease-causing variants reported in HGMD®, in ClinVar or in CentoMD® are evaluated. The investigation for relevant variants is focused on coding exons and flanking +/-10 intronic nucleotides of genes with clear gene-phenotype evidence (based on OMIM® information). All potential patterns for mode of inheritance are considered. In addition, provided family history and clinical information are used to evaluate identified variants with respect to their pathogenicity and disease causality. Variants are categorized into five classes (pathogenic, likely pathogenic, VUS, likely benign, and benign) along ACMG guidelines for classification of variants. All relevant variants related to the phenotype of the patient are reported. CENTOGENE has established stringent quality criteria and validation processes for variants detected by NGS. Variants with low sequencing quality and/or unclear zygosity are confirmed by orthogonal methods. Consequently, a specificity of > 99.9% for all reported variants is warranted. Mitochondrial variants are reported for heteroplasmy levels of 15% or higher. The copy number variation (CNV) detection software has a sensitivity of more than 95% for all homozygous/hemizygous and mitochondrial deletions, as well as heterozygous deletions/duplications and homozygous/hemizygous duplications spanning at least three consecutive exons. For the uniparental disomy (UPD) screening, a specific algorithm is used to assess the well-known clinically relevant chromosomal regions (6q24, 7, 11p15.5, 14q32, 15q11q13, 20q13 and 20).

ANALYSIS STATISTICS

CentoXome® Solo

Targeted nucleotides covered	≥ 20x	99.27%

LIMITATIONS

CentoXome® Solo

The genetic results are interpreted in the context of the provided clinical findings, family history, and other laboratory data. Only variants in genes potentially related to the proband's medical condition are reported. Misinterpretation of results may occur if the provided genetic data or patient information is inaccurate and/or incomplete. If the obtained genetic results are not compatible with the clinical findings, additional testing should be considered.

The genes with mapping issues in GRCh37/hg19 genome assembly, the non-protein-coding disease-associated genes, and approximately 0.2 Mb of genomic regions that are hard to sequence by current enrichment technology and are without evidenced relevance for monogenic disorders, are excluded from this analysis. More complex genetic events such as inversions, translocations, and repeat expansions, are not analyzed in this test. The UPD detection is a screening method, and therefore false-positive and false-negative results may occur. In addition, due to technology limitations, certain regions may be poorly covered, or not covered at all. In these regions and others encompassing repetitive, high-homology (such as pseudogene homology), and GC-rich sequences, relevant variants can be missed. Extremely low-coverage calls (homo/hemizygous or heterozygous calls with less than three or four reads, respectively) are expected to be artifacts based on our extensive validations and are consequently not considered during the analysis. Heterozygous CNVs spanning less than three exons cannot reliably be detected, are therefore excluded from routine analysis, and will only be inspected and reported upon medical or technical indication. The CNV detection sensitivity is decreased for repetitive and homologous regions, such as pseudogenes. Mitochondrial variants with heteroplasmy levels below 15% may not be detected. It is expected that lower quality samples (prenatal, product of conception, blood from patients with hematologic disorders, and highly degraded DNA) may generate lower quality NGS data; in these cases, CNV analysis and/or mitochondrial genome analysis may not be possible to perform. Potential aberrant splicing is assessed with splice prediction tools. Intronic variants that are beyond 10 nucleotides from exon-intron boundaries are not considered for aberrant splicing analysis, with the exception of known pathogenic splicing variants evidenced by external sources.

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ADDITIONAL INFORMATION

This test was developed, and its performance was validated, by CENTOGENE. 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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