POSITIVE RESULT
Pathogenic variants identified

INTERPRETATION
Two heterozygous pathogenic variants were identified in the BBS1 gene in compound heterozygous state.

The genetic diagnosis of autosomal recessive Bardet-Biedl syndrome type 1 is confirmed.

RECOMMENDATIONS
- Genetic counselling is recommended.
RESULT SUMMARY

<table>
<thead>
<tr>
<th>GENE</th>
<th>VARIANT COORDINATES</th>
<th>ZYGOSITY</th>
<th>IN SILICO PARAMETERS*</th>
<th>ALLELE FREQUENCIES**</th>
<th>TYPE AND CLASSIFICATION***</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBS1</td>
<td>Chr11(GRCh37):g.66278561G&gt;A &lt;br&gt; NM_024649.4:c.124+1G&gt;A</td>
<td>Het</td>
<td>PolyPhen: N/A &lt;br&gt; Align-GVGD: N/A &lt;br&gt; MutationTaster: N/A &lt;br&gt; Conservation: nt weak &lt;br&gt; 3/3 likely splice effect</td>
<td>gnomAD: - &lt;br&gt; ESP: -&lt;br&gt; 1000 G: - &lt;br&gt; CentoMD: -</td>
<td>Substitution &lt;br&gt; Pathogenic (class 1)</td>
</tr>
<tr>
<td></td>
<td>Intron 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BBS1</td>
<td>Chr11(GRCh37):g.66291105C&gt;T &lt;br&gt; NM_024649.4:c.951+58C&gt;T</td>
<td>Het</td>
<td>PolyPhen: N/A &lt;br&gt; Align-GVGD: N/A &lt;br&gt; MutationTaster: N/A &lt;br&gt; Conservation: nt moderate &lt;br&gt; 2/3 likely splice effect</td>
<td>gnomAD: - &lt;br&gt; ESP: -&lt;br&gt; 1000 G: - &lt;br&gt; CentoMD®: 0.000095</td>
<td>Substitution &lt;br&gt; Pathogenic (class 1)</td>
</tr>
</tbody>
</table>

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

**BBS1, c.124+1G>A**

The BBS1 variant c.124+1G>A is predicted to disrupt the highly conserved donor splice site of exon 2. Thus, a skip of exon 2 is very likely. According to HGMD Professional 2018.4, this variant has previously been described as disease causing for Bardet-Biedl syndrome by Harville et al., 2010 (PMID: 19797195) and Abu Safieh et al., 2010 (PMID: 19858128). This variant was detected in the mother in heterozygous state. It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

**BBS1, c.951+58C>T**

The BBS1 variant c.951+58C>T is predicted to create an intronic donor splice site, which is likely to cause a shift in the reading frame and a loss of function of the BBS1 protein. According to HGMD Professional 2017.3, this variant has previously been described as disease causing for Bardet-Biedl syndrome by Abu Safieh et al., 2010 (PMID: 19858128) and Scheidecker et al., 2015 (PMID: 25982971). This variant was detected in the father in heterozygous state. It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in the BBS1 gene are known to cause Bardet-Biedl syndrome type 1 (OMIM®: 209900). Bardet-Biedl syndrome (BBS) is an autosomal recessive and genetically heterogeneous ciliopathy. It is characterized by rod-cone dystrophy, truncal obesity, postaxial polydactyly, cognitive impairment, male hypogonadotropic hypoogonadism, complex female genitourinary malformations, and renal abnormalities. The visual prognosis for children with BBS is poor. Night blindness is usually evident by age seven to eight years. Birth weight is usually normal, but significant weight gain begins within the first year and becomes a lifelong issue for most individuals. A majority of individuals have significant learning difficulties. Renal disease is a major cause of morbidity and mortality (PMID: 20301537).

INCIDENTAL FINDINGS

We did not detect any class 1 or 2 variants in the genes for which incidental findings are reported based on the ACMG guidelines.
ANALYSIS STATISTICS WES

<table>
<thead>
<tr>
<th>% TARGET NUCLEOTIDES COVERED</th>
<th>≥ 10X</th>
<th>≥ 20X</th>
<th>≥ 50X</th>
</tr>
</thead>
<tbody>
<tr>
<td>0X</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>99.87</td>
<td>99.42</td>
<td>94.87</td>
</tr>
</tbody>
</table>

CENTOGENE VARIANT CLASSIFICATION (BASED ON ACMG RECOMMENDATIONS)

- **Class 1** – Pathogenic
- **Class 2** – Likely pathogenic
- **Class 3** – Variants 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

Double stranded DNA capture baits against approximately 36.5 Mb of the human coding exome (targeting >98% of the coding RefSeq and Gencode v28 regions, which was obtained from the human genome build GRCh37/hg19 on May 2018) were used to enrich target regions from fragmented genomic DNA with the Twist Human Core Exome Plus kit. 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, variant calling and annotation, and comprehensive variant filtering is applied. All disease-causing variants reported in HGMD®, in ClinVar and in CentoMD® as well as all variants with minor allele frequency (MAF) below 1% in gnomAD database are considered. The investigation for relevant variants is focused on coding exons and flanking +/-20 intronic bases. All potential modes of inheritance patterns are considered. In addition, provided family history and clinical information are used to evaluate identified variants with respect to their pathogenicity and causality, and are categorized into classes 1 - 5 (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 NGS. Low quality single nucleotide variants and all relevant deletion/insertion variants are confirmed by Sanger sequencing. Consequently, we warrant a specificity of >99.9% for all reported variants.

LIMITATIONS

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 information is inaccurate and/or incomplete. If the obtained genetic results do not concur with the clinical findings, additional testing should be considered. Complex genetic events such as copy number variants, inversions, translocations and repeat expansions, may not be reliably detected with Exome Sequencing. In addition, due to technology limitations, certain regions may be either not or poorly covered. In these regions variants cannot be confidently detected. 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 consequently are not considered during the analysis.

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.

In line with ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing (Genetics in Medicine, 2016), we report incidental findings, i.e. pathogenic variants (class 1) and likely pathogenic variants (class 2) only in the recommended genes for the recommended phenotypes.

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 (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...
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